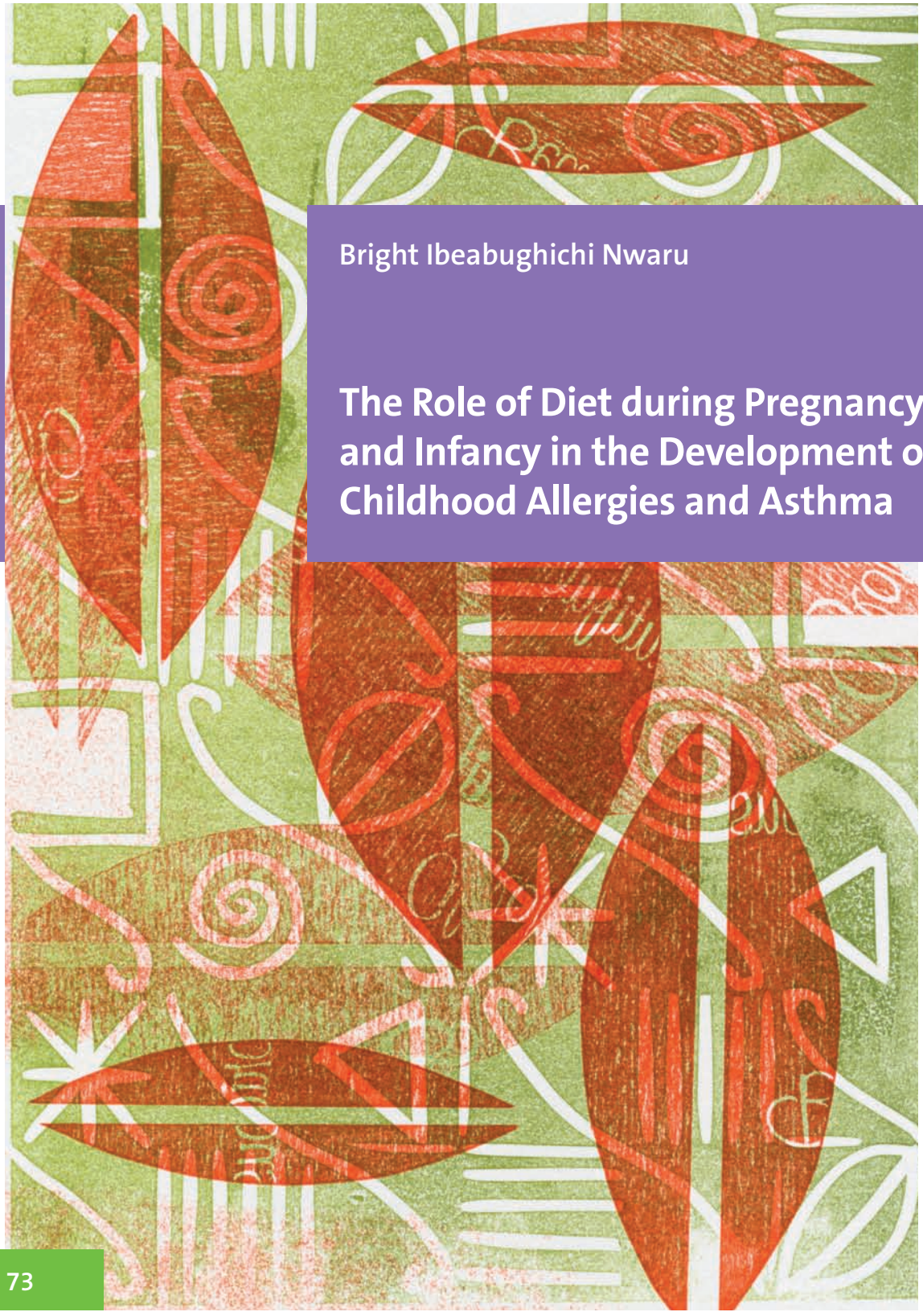
NATIONAL INSTITUTE
FOR HEALTH AND WELFARE



RESEARCH

Bright Ibeabughichi Nwaru

The Role of Diet during Pregnancy and Infancy in the Development of Childhood Allergies and Asthma

RESEARCH 73

Bright Ibeabughichi Nwaru

The Role of Diet during Pregnancy and Infancy in the Development of Childhood Allergies and Asthma

ACADEMIC DISSERTATION

To be presented with the permission of the board of the School of Health Sciences, University of Tampere, for public examination in the auditorium of School of Health Sciences, Medinsiinarinkatu 3, Tampere, on January 27, 2012, at 12 o'clock

School of Health Sciences, University of Tampere, Finland
and National Institute for Health and Welfare, Helsinki, Finland

University of Tampere

Tampere 2012

NATIONAL INSTITUTE
FOR HEALTH AND WELFARE

Cover photo:

ISBN 978-952-245-573-4 (printed)
ISSN 1798-0054 (printed)
ISBN 978-952-245-574-1 (pdf)
ISSN 1798-0062 (pdf)

Juvenes Print - Tampere University Print
Tampere, Finland 2012

Supervised by
Professor Suvi M. Virtanen, MD, PhD
Nutrition Unit, Department of Lifestyle and Participation
National Institute for Health and Welfare
Helsinki, Finland
School of Health Sciences
University of Tampere
Tampere, Finland

Professor Minna Kaila, MD, PhD
Hjelt Institute
University of Helsinki
Helsinki, Finland

Reviewed by
Docent Petri Kulmala
Hospital for Children and Adolescents
University of Oulu
Oulu, Finland

Docent Kirsi Laitinen
Functional Foods Forum
University of Turku
Turku, Finland

Opponent
Docent Mika Mäkelä
Hospital District of Helsinki and Uusimaa
Helsinki, Finland

Dedicated to
Chinomnso and Chinazam

Abstract

Bright I. Nwaru. The Role of Diet during Pregnancy and Infancy in the Development of Childhood Allergies and Asthma. National Institute for Health and Welfare (THL). Research 73. 125 pages. Helsinki, Finland 2012.
ISBN 978-952-245-573-4 (printed); ISBN 978-952-245-574-1 (pdf)

Over the last decade, the prevalence of allergies and asthma in childhood has increased, particularly in industrialized societies. In Finland, the cumulative incidence of eczema is one of the highest in the world. Allergies and asthma are ailments with strong genetic predisposition, manifesting through familial history. The genetic predisposition, nonetheless, has failed to explain the increasing trend. Among other environmental factors, prenatal and early life dietary exposures have been hypothesized to play a role in the development of allergies and asthma in childhood. This proposition has constituted an area of intense research at present. Diet is a modifiable exposure, and understanding its role in the occurrence of allergies and asthma in children may provide clues to early dietary interventions. The main aim of this thesis was to investigate the effect of maternal diet during pregnancy (Studies I & IV) and the child's diet during infancy (Study II) on the risk of allergies and asthma in childhood. The Finnish version of the ISAAC questionnaire on asthma was validated against the Social Insurance Institution database on asthma (Study III).

This study was done on the basis of the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Nutrition Study, which started in September 1996 in Oulu (Northern Finland) and October 1997 in Tampere (Southern Finland) University hospital areas. The pregnancy component of the nutrition study started in October 1997. Altogether 4065 children born between September 2, 1996 and September 5, 2004 were invited to the allergy component of the DIPP Nutrition Study at the age of 5 years; 3737 (92%) of these participated. All the necessary dietary and allergy data were available for 931 children in Study I, for 994 in Study II, for 2236 in Study III, and 2441 in Study IV. The validity of the ISAAC questionnaire was esti-

mated with the sensitivity, specificity, positive (PPV) and negative (NPV) predictive values, and the Youden's index. Logistic regression was used in Studies I and II, while Cox hazards regression was used in Study IV.

The sensitivity (0.98, 95% CI 0.92-0.99), specificity (0.98, 95% CI 0.97-0.98), and NPV (1.00, 95% CI 1.00-1.00) of the ISAAC questionnaire were excellent; the PPV (0.63, 95% CI 0.55-0.71) was above average. In adjusted models that included several potential confounding variables, maternal consumption of total fruits (OR 1.36, 95% CI 109-1.70) and citrus fruit (OR 1.14, 95% CI 1.05-1.25) was associated with increased risk of inhalant allergic sensitization in the offspring at the age of 5 years. Maternal intake of vitamin D (OR 0.56, 95% CI 0.35-0.91) and magnesium (OR 0.78, 95% CI 0.62-0.97) was inversely associated with sensitization to food allergens and atopic eczema, respectively. When all complementary foods were studied together and adjusted for potential confounding variables, late introduction of eggs (>10.5 months), oats (>5 months), and wheat (>6 months) was directly associated with positivity for IgE antibodies for food and inhalant allergens; whereas late introduction of eggs and wheat increased the risk for cow's milk and wheat IgE antibodies.

The results from this prospective birth cohort study provide evidence for a potential role of prenatal and early life dietary exposures in the development of allergies and asthma in childhood. The observed protective effect of vitamin D is consistent with previous studies and may require a confirmation in a randomized trial setting. Further studies are required to corroborate the observed protective association of prenatal intake of magnesium in the occurrence of allergies in children. The optimal length of exclusive breastfeeding for the prevention of allergies and asthma in childhood remains to be determined. For the introduction of complementary foods, it could be concluded that they should not be delayed beyond six months. However, the evidence is still accumulating on the specific time prior to six months would be the optimal time. Although current findings have mainly emanated from observational epidemiological studies, they, in sum, indicate that there may be need for a careful review of current recommendations on infant feeding for the prevention of

allergies and asthma. Ethical concerns may inhibit carrying out randomized controlled trial to confirm these findings, since breastfeeding cannot be randomized.

Studies in this area will endeavor to overcome the methodological challenges in relating early life dietary exposures to the occurrence of allergies and asthma in childhood. These challenges include more precise measurement of prenatal and early life dietary intakes and a more uniform diagnosis and definition of allergic outcomes and asthma in children, as well as examining their seasonal variations; adjusting for important potential confounding factors; and disentangling the independent effects of maternal and infant's diet on the risk of allergies and asthma in children. Beyond the potential roles of individual foods, nutrients, and overall dietary patterns, extending research in this area to incorporate the larger role of the food supply chain (from production to consumption), examining possible contaminants in the process, may give a more comprehensive understanding of the role of diet in the increasing prevalence of allergies and asthma in children.

Keywords: allergies, asthma, breastfeeding, children, complementary foods, epidemiology, nutrition, pregnancy, validation

Tiivistelmä

Bright I. Nwaru. The Role of Diet during Pregnancy and Infancy in the Development of Childhood Allergies and Asthma. Terveyden ja hyvinvoinnin laitos (THL). Tutkimus 73. 125 sivua. Helsinki, Finland 2012.
ISBN 978-952-245-573-4 (painettu); ISBN 978-952-245-574-1 (pdf)

Lapsuusiän allergioiden ja astman esiintyvyys on lisääntynyt viimeisen vuosikymmenen aikana erityisesti teollistuneissa maissa. Suomessa allergisten ihottumien (ekseema) kumulatiivinen ilmaantuvuus on yksi maailman suurimmista. Allergiat ja astma ovat sairauksia, joihin liittyy voimakas perinnöllinen alttius. Niiden esiintyvyyden kasvua ei ole kuitenkaan pystytty selittämään perinnöllisellä tautialttiudella. Muiden ympäristötekijöiden ohella äidin raskaudenaikaisen ruokavalion ja lapsen varhaisiän ruokavalion on oletettu vaikuttavan allergioiden ja astman kehittymiseen lapsilla. Tämä oletus on johtanut siihen, että varhaisen ravitsemuksen yhteyttä allergioihin ja astmaan tutkitaan tänä päivänä tiiviisti. Ruokavalio on altiste, jota voidaan muokata ja sen merkityksen ymmärtäminen allergioiden ja astman kehittymisessä voi avata mahdollisuuksia varhaisiän ruokavaliointerventioihin. Tämän väitöskirjan päätavoite oli selvittää äidin raskaudenaikaisen ruokavalion (Tutkimukset I & IV) ja lapsen imeväisiän ruokavalion (Tutkimus II) vaikutusta lapsuusiän allergisten sairauksien riskiin. Suomalainen versio ISAAC-kyselystä validoitiin Kansaneläkelaitoksen rekisteristä saatuja tietoja (astmalääkityksen erityiskorvattavuus) vasten (Tutkimus III).

Tutkimuksen aineistona käytettiin suomalaisen tyypin 1 diabeteksen ennustaminen ja ehkäisy-tutkimuksen (DIPP) ravintotutkimuksen aineistoa. DIPP-ravintotutkimus alkoi syyskuussa 1996 Oulun ja lokakuussa 1997 Tampereen yliopistollisen sairaalan alueella. Äidin raskaudenaikaisen ravitsemuksen aineiston keruu alkoi lokakuussa 1997. DIPP-ravintotutkimuksen allergiaosioon kutsuttiin 5 vuoden iässä 4065 lasta, jotka olivat syntyneet 2.9.1996-5.9.2004. Allergiatutkimukseen osallistui 3737 lasta (92% kutsutuista). Tarvittavat ruokavalio- ja allergiatiedot

olivat saatavissa 931 lapselta (Tutkimus I), 994 lapselta (Tutkimus II), 2236 lapselta (Tutkimus III) ja 2441 lapselta (Tutkimus IV). ISAAC-kyselylomakkeen validiteetti arvioitiin herkkyyden (sensitiivisyys), tarkkuuden (spesifisyys), positiivisen (PPV) ja negatiivisen (NPV) ennustearvon avulla sekä Youdenin indeksillä. Logistista regressiota käytettiin tutkimuksissa I ja II ja Coxin regressiota tutkimuksessa IV.

ISAAC-kyselyn sensitiivisyys (0.98, 95%:n luottamusväli 0.92-0.99), spesifisyys (0.98, 0.97-0.98) ja NPV (1.00, 1.00-1.00) olivat erinomaiset. PPV (0.63, 0.55-0.71) oli keskimääräistä parempi. Vakioiduissa malleissa, jotka sisälsivät useita mahdollisia sekoittavia tekijöitä, äidin hedelmien (kaikki hedelmät) (OR 1.36, 95%:n luottamusväli 1.09-1.70) ja sitrushedelmien (OR 1.14, 1.05-1.25) käyttö olivat yhteydessä lapsen hengitystieallergeeneille herkistymisen kasvaneeseen riskiin viiden vuoden iässä. Äidin D-vitamiinin saanti oli käänteisessä yhteydessä lapsen ruoka-allergeeneille herkistymiseen (OR 0.56, 0.35-0.91) ja äidin magnesiumin saanti oli käänteisessä yhteydessä lapsen atooppiseen ekseemaan (OR 0.78, 0.62-0.97). Kun kaikki lapsen lisäravinnon aloitusiät tutkittiin yhdessä ja vakioitiin mahdollisilla sekoittavilla tekijöillä, myöhäinen kananmunan (>10.5 kk), kauran (>5 kk) ja vehnän (>6 kk) aloitusikä olivat suorassa yhteydessä lapsen ruoka- ja hengitystieallergeeneille herkistymiseen, kun taas kananmunan ja vehnän myöhäinen aloitusikä lisäsivät lapsen riskiä lehmänmaidolle ja vehnälle herkistymiseen.

Tämän prospektiivisen syntymäkohorttitutkimuksen tulokset antavat näyttöä äidin raskaudenaikaisen ja lapsen varhaisiän ruokavalion mahdollisesta yhteydestä lapsuusiän allergioiden ja astman kehittymiseen. Havaittu D-vitamiinin suojaava vaikutus on yhdenmukainen aiempien tutkimustulosten kanssa ja sen vahvistaminen satunnaistetussa koeasetelmassa voisi olla tarpeen. Äidin raskaudenaikaisen magnesiumin saannin suojaava vaikutus lapsen allergioiden kehittymiseen vaatii lisätutkimuksia. Lapsuusiän allergioita ja astmaa ehkäisevän täysimetyksen optimaalinen kesto on edelleen määrittelemättä. Johtopäätöksenä tästä tutkimuksesta voidaan todeta, että lisäruokien aloittamisen ei tulisi pitkittyä yli lapsen kuuden kuukauden iän. Toisaalta tutkimusnäytön kertyminen siitä, mikä olisi optimaalinen aika aloittaa lisäruokien antaminen lapselle, on myös vielä kesken. Vaikka nykyiset tutkimuslöydökset ovat peräisin pääasiassa epidemiologisista seurantatutkimuksista, ne yhdessä

viittaavat siihen, että nykyisiä allergioiden ja astman ehkäisemiseen tähtääviä imeväisruokinnan suosituksia saattaisi olla aiheellista arvioida huolellisesti uudelleen. Satunnaistetun kontrolloidun kokeen toteuttaminen tulosten vahvistamiseksi on eettisesti ongelmallista, koska imetystä ei voi asettaa satunnaistettavaksi.

Lapsuusiän allergioita ja astmaa koskevissa tutkimuksissa pyritään ratkaisemaan myös menetelmällisiä haasteita. Näitä ovat muun muassa varhaisen ravitsemuksen tarkempi mittaaminen, allergia- ja astmavasteiden yhtenäisempi määrittely ja diagnosointi sekä näiden vuodenaikaisvaihtelun tarkastelu, tärkeiden mahdollisten sekoittavien tekijöiden huomioon ottaminen, ja äidin ja lapsen ruokavalioiden itsenäisten vaikutusten erittely arvioitaessa ravitsemuksen yhteyttä lapsuusiän allergioiden ja astman riskiin. Yksittäisten ruokien, ravintoaineiden ja kokonaisruokavalion vaikutusten lisäksi, tutkimuksen laajentaminen ottamaan huomioon koko ruoan tuottaja-kuluttaja-ketju voisi tuottaa kokonaisvaltaisemman ymmärryksen ruokavalion merkityksestä lasten allergioiden ja astman yleistymisessä.

Contents

LIST OF ORIGINAL CONTRIBUTIONS

I Nwaru BI, Ahonen S, Kaila M, Erkkola M, Haapala A-M, Kronberg-Kippilä C, Veijola R, Ilonen J, Simell O, Knip M, Virtanen SM. Maternal diet during pregnancy and allergic sensitization in the offspring by 5 yrs of age: a prospective cohort study. *Pediatric Allergy and Immunology* 2010; 21: 29-37.

II Nwaru BI, Erkkola M, Ahonen S, Kaila M, Haapala A-M, Kronberg-Kippilä C, Salmelin R, Veijola R, Ilonen J, Simell O, Knip M, Virtanen SM. Age at the introduction of solid foods during the first year and allergic sensitization at age 5 years. *Pediatrics* 2010; 125: 50-59.

III Nwaru BI, Lumia M, Kaila M, Luukkainen P, Tapanainen H, Erkkola M, Ahonen S, Pekkanen J Klaukka T, Veijola R, Simell O, Knip M, Virtanen SM. Validation of the Finnish ISAAC questionnaire on asthma against anti-asthmatic medication reimbursement database in 5-year-old children. *The Clinical Respiratory Journal* 2011; 5: 211-218.

IV Nwaru BI, Erkkola M, Ahonen S, Kaila M, Kronberg-Kippilä C, Ilonen J, Simell O, Knip M, Veijola R, Virtanen SM. Intake of antioxidants during pregnancy and the risk of allergies and asthma in the offspring. *European Journal of Clinical Nutrition* 2011; 65: 937-943.

1 INTRODUCTION

Over the past decades, the prevalence of allergies and asthma in children has increased, particularly in industrialized societies (Seaton *et al* 1994; Becker 2000; Devereux 2006; Pearce & Douwes 2006). For instance, although with notable variations across regions of the world, a 0.18% and 0.28% increase in ever asthma was seen among children age 6-7 and 13-14 years old, respectively during a period of 5-9 years (Pearce *et al* 2007). Although initial speculation for the increase was a consequence of increased awareness, classification, and diagnosis of allergic symptoms, other evidence points to a real increase (Seaton *et al* 1994). In Finland, between 1966 and 1989, there was almost a two-fold increase in asthma among conscripts to the Finnish defense force (Haahtela *et al* 1990). The authors ruled out improved diagnosis as an explanation for the rise, since the increase coincided with a concomitant rise in service exemptions and discharges due to asthma. Another Finnish study also showed almost a three-fold increase in asthma among children aged 0-4 years during a 20-year period (Malmström *et al* 2000). For the most part, the causes of the increase in prevalence in allergies and asthma have remained unclear, and are currently the focus of several epidemiologic studies globally (Asher *et al* 1995; Enarson 2005).Lorem ipsum dolor sit amet, consectetueradipiscing elit, sed diam nonummy nibh euismod tincidunt ut laoreet doloremagna aliquam erat volutpat.

Allergic disorders are believed to be a result of an induced shift in the balance between the Th1 and Th2 cytokines which favor Th2 dominance (Kay, 2001; Li-Weber *et al* 2002). For asthma and other allergies like wheeze, oxidative free radicals are released in this process, resulting in inflammatory damages to the lung (Seaton *et al* 1994; Li-Weber *et al* 2002). A common feature of allergic subjects is the accumulation of immunoglobulin E (IgE)-mediated immune responses to allergen exposures, resulting in allergic sensitization (Wahn *et al* 1997; Kulig *et al* 1999). The build-up to the sensitization of the IgE is believed to be initiated very early in life, possibly, as early as *in utero* (Devereux *et al* 2002; van Gool *et al* 2004; Kumar

2008). Consequently, allergies and asthma are diseases with strong genetic and familial predispositions (Devereux *et al* 2002; van Gool *et al* 2004).

There is a general consensus that the changes in genetic susceptibility of the population can only explain a portion of the increasing prevalence of childhood allergies and asthma (Seaton *et al* 1994; Becker 2000). Other propositions have been presented to explain the increase, including changes in exogenous environmental factors (Becker 2000; Kumar 2008; Dietert & Zelikoff 2008) and the so-called 'hygiene hypothesis' (i.e. decreased or absence of microbial exposures in early life) (Strachan 1989; Matricardi & Yazdanbakhsh 2003; Platts-Mills *et al* 2005). In addition to an active research to understand the contribution of these factors, increased attention has recently been focused on the role of diet in the occurrence of allergies (Devereux & Seaton 2005; Novak & Leung 2005; Devereux 2007; Schneider *et al* 2007; Litonjua 2008; Vries & Howie 2009). It has been suggested that the increase in allergies and asthma corresponded with observable changes in the diet of industrialized societies, from traditional to more processed diet (Seaton *et al* 1994; Black & Sharpe 1997; Fogarty & Briton 2000; Devereux 2007). Considering that allergic predisposition is already initiated very early in life, particular attention has been paid to the effect of diet during the prenatal and early life on its subsequent development later in life (Devereux & Seaton 2005; Devereux 2007; Vries & Howie 2009; Robison & Kumar 2010).

Diet is a modifiable exposure and understanding the role of early life dietary exposure in the development of allergies in children may provide an important measure to early dietary intervention (Devereux 2007). Presently a number of birth cohorts are being implemented around the world to investigate the effect of diet during early life on the occurrence of allergies and asthma in childhood. The main objective of this thesis was to evaluate whether dietary exposures during pregnancy and early infancy may play a role in the occurrence of allergies and asthma during early childhood.

2 REVIEW OF THE LITERATURE

2.1 Epidemiology of allergies and asthma in childhood

2.1.1 Definitions, mechanisms, and assessment of allergies and asthma

Basically, allergy is defined as the hypersensitivity of the immune system usually in response to exposure to harmless environmental substances called antigens/allergens, which are mainly found in house dust mites, pets, pollen, insects, moulds, foods and some medicines (Johansson et al 2004; Grant & Horner 2006; Burks & Palmer 2008). In the event of exposure to these antigens, the immune system invites the IgE, a protective antibody, in majority of the cases, to fight the invading substances (Burks & Palmer 2008). Mast cells, including histamines, are released in this process into the tissues and blood. The irritating nature of these cells causes itching and swelling (inflammation), which can lead to the narrowing of the lung airway (Burks & Palmer 2008). This whole process ends with the presentation of the common medical conditions, such as allergic rhinitis (which usually occurs in the nose and /or eyes), eczema (on the skin), wheeze and asthma (in the lungs) (Burks & Palmer 2008). Thus, allergies and asthma are ailments with strong immuno-modulatory effects, involving inappropriate responses of the Th-2 cell phenotype to common allergens (Abbas *et al* 1996; Georas *et al* 2005).

The diagnosis and assessment of allergies and asthma varies by age, but in clinical practice the most common procedures in children include a careful examination of the case history by a physician, determination of the IgE sensitization in the blood, allergen challenges, histamine release and patch tests (Eigenmann 2005; Grant & Horner 2006; Burks & Palmer 2008), and additionally for asthma, pulmonary function and bronchial provocation tests. Due to the fact that allergies manifest with differing phenotypes and, sometimes, may present with similar symptoms during childhood, valid diagnosis and case definition may pose tremendous challenge.

Despite the value of clinical diagnosis as a more objective assessment of allergies and asthma in children (Pekkanen & Pearce 1999; Marklund *et al* 1999; Remes *et al* 2002; Wordemann *et al* 2006), it has also been subject to under-diagnosis (Moth *et al* 2007) and some differences in clinical practice (Csonka *et al* 2000; Ward *et al* 2004). As a result of these challenges, assessment of childhood allergies and asthma for epidemiological research purposes has been mostly undertaken through parental-reported questionnaire (Pekkanen & Pearce 1999; Remes *et al* 2002; Wordemann *et al* 2006).

Due to lack of standardized methodology for questionnaire assessment of childhood allergies and asthma, the International Study of Asthma and Allergies in Childhood (ISAAC) was established (Asher *et al* 1995). It is a worldwide initiation to understand the contribution epidemiology can make in understanding the etiology of allergies and asthma in children through a standardized case-definition of the diseases. It constitutes three phases: Phase I assesses the prevalence and severity of allergies in children; Phase II investigates the potential etiological risk factors suggested in Phase I; Phase III aims at repetition of Phase I after a 3-year period. Subjects in the ISAAC study are 6-7 year olds with parental-reported allergic symptoms and 13-14 year olds who reported their own allergic symptoms (The ISAAC Manual 1993; Asher *et al* 1995). The ISAAC questionnaire modules have been modified and implemented in several countries across the world.

2.1.2 The prevalence and worldwide burden of allergies and asthma

Based on recent ISAAC data, the prevalence of allergies and asthma is seen to have increased between the Phase I and Phase III data collection periods (about 5-9-year period), although with great regional variations (Pearce *et al* 2007). For instance, the current global prevalence of asthma in the 6-7- and 13-14–year-old children is about 11% and 14%, respectively, which respectively represent a 0.18% and 0.28% increase during the last 5-9 years (Pearce *et al* 2007). Similar trends have also been reported for other allergies, such as allergic rhinitis (Asher *et al* 2006; Björkstén *et*

al 2008) and eczema (Asher *et al* 2006). While the attribution of the increasing prevalence of allergies and asthma as a phenomenon of the industrialized societies (Douwes & Pearce 2002; Pearce & Douwes 2006; Devereux 2006) may be true in terms of absolute comparison of the prevalence across regions (Patel *et al* 2008; Aït-Khaled *et al* 2009), there are some evidence that the prevalence is also increasing in several developing settings (Pearce *et al* 2007; Björkstén *et al* 2008; Patel *et al* 2008; Aït-Khaled *et al* 2009). For instance, some gradual increasing trends of asthma and wheeze were observed in some low and middle income countries where the prevalence was previously known to have been low (Pearce *et al* 2007; Björkstén *et al* 2008). In the case of rhinoconjunctivitis, it was also shown that, although the prevalence was higher in high income countries than in developing settings, the prevalence of severe symptoms and the co-morbidity of the allergies were greater in less affluent settings (Aït-Khaled *et al* 2009).

As chronic ailments, allergies and asthma pose great challenge, not only to the individual child and the family, but also to the health care system and the economy at large in terms of cost burdens. For instance, in the US alone it was estimated that the annual cost for treatment of asthma (Devereux 2006) and food allergies (Patel *et al* 2011) is about 11 billion and half a billion dollars, respectively. In the UK, the corresponding figure for asthma is about 2.3 billion pounds, and out of the 5.2 million people treated for asthma, 1.1 million are children (Devereux 2006). In Latin America, the cost for unscheduled health care for asthmatics has been shown to present a great burden to the national economy (Neffen *et al* 2010). This same trend has been shown to be similar in other contexts (Schoenwetter *et al* 2004; Kim *et al* 2010). With the increasing prevalence of the diseases in less affluent countries, which are still fighting the cost of other impending diseases, it may be difficult to project the absolute burdens childhood allergies and asthma may pose to the society. In view of this, intense efforts have been launched in recent times to search for the potential risk factors causing the increase, particularly the environmental and lifestyle exposures, so that early preventive strategies can be initiated.

2.1.3 Childhood allergies and asthma in Finland

In Finland, allergies and asthma represent one of the most common chronic diseases in children. Examination of hospital admission for asthma during a 20-year period showed almost a three-fold increase in admission rate among children aged 0-4 years (Malmström *et al* 2000). The current prevalence of eczema in Finnish children is estimated to be about 15-20%, being one of the highest in the world (Lethtonen *et al* 2003; Kaila *et al* 2009), while 5% of Finnish children is said to have asthma (Haahtela *et al* 2008). Sensitization to any allergen among school aged children was estimated at 43% in 2003 (von Hertzen *et al* 2006). A recent study of a birth cohort established in 1985 indicated that by the age of 5 years, allergy is diagnosed in about 9% of children, and that allergic children are about 8 and 7 times more likely to have allergy and asthma in adolescence, respectively (Kaila *et al* 2009).

Although signs of reduction in the prevalence of allergies and asthma in Finland have not been seen, it has been observed that days of hospitalization due to asthma have drastically reduced (Haahtela *et al* 2006; Haahtela *et al* 2008). It is also suggested that, against a 1993 prediction of increased cost burden attributable to asthma by the year 2005, the actual spending for asthma treatment was halved by that year (Haahtela *et al* 2008). With the aim of reducing the burden of allergies, the Finnish Allergy Programme 2008-2018 was introduced. It is hoped that by the end of this programme, cogent preventive strategies for the development of allergies would be enforced; it is expected that there would be an increase in the tolerance against allergens; the diagnostic procedures of allergies would be improved; decreased work-related allergies, severity of allergies, and the cost associated with allergy treatments (von Hertzen L & Haahtela 2005; Haahtela *et al* 2008).

2.2 Early life origins of allergies and asthma

The concept of 'fetal programming' has received wide attention, which explains that environmental exposures during the prenatal stage can produce both structural and functional effects that do not only manifest in early life but may persist even for the

whole lifespan (Barker 1998; Barnes 2008; de Vries & Howie 2009). There is accumulating evidence that the programming and development of the immune system may already be shaped by the *in utero* environment (Barker 1998; Calder *et al* 2006; Barnes 2008; de Vries & Howie 2009).

Although the understanding of the timing of the origin of the encounter between the immune system and allergens remains unclear, evidence suggest that the initiation may already manifest *in utero* (Jones *et al* 1996; Devereux 2007). It has been suggested that circulating T-cells can already be detected from 15 weeks of gestation, and stimulating them with in vitro manifest proliferation that is detectable from 22 weeks (Jones *et al* 1996). In cord blood, allergy precursors such as emerging cytokines, IgE levels, and a number of T lymphocytes have been shown to predict acute lower respiratory diseases and the risk of allergies later in infants (Spinozzi *et al* 2001; Maritz *et al* 2005; Ly *et al* 2007; Scirica *et al* 2007). These set of evidence are indicative of the importance of the prenatal and infant periods as the stages of life that may be critical for the initiation of allergic disorders. Consequently, the increasing attention of the role of early life diet in the development of allergies and asthma in children is well-placed.

2.2.1 Risk factors for allergies and asthma in childhood

The most important risk factor for the development of allergies is genetic predisposition, manifesting through family hereditary lines (Becker 2000). Consistently, studies have shown that children with family history of allergies are more likely to develop allergies and asthma than children from non-allergic families (Calder *et al* 2006). However, the particular genes responsible for the development of allergies and asthma are still being investigated (Becker 2000; Moffatt *et al* 2010). So far, genetic predispositions have failed to explain most of the current increase in allergies and asthma in children (Seaton *et al* 1994; Becker 2000).

Consequently, other environmental and lifestyle exposures in early life have been identified to contribute as risk factors for childhood allergy. Among the prominent

factors so far identified, the so called 'hygiene hypothesis' has received increased attention (Strachan 1989; Calder *et al* 2006). The 'hygiene hypothesis' proposes that the absence of microbial exposures in early life, such as helminth infection, exposure to endotoxins, exposure to pets, and growing up in a farm, may increase the risk for allergies later in childhood (Matricardi & Yazdanbakhsh 2003; Platts-Mills *et al* 2005; Gern 2011).

Although maternal smoking during pregnancy has been identified as one of the important risk factors for the onset of allergies and asthma in the offspring (Cook & Strachan 1997; Devereux *et al* 2002; Hagendorens *et al* 2005; Dietert & Zelikoff 2008), some studies have found it to rather decrease the risk (Kuyucu *et al* 2004), while others have reported no association (Purvis *et al* 2005). Attendance to day care (Hagendorens *et al* 2005; Morais-Almeida *et al* 2007) and owing a pet at home by the first year of life (Ownby *et al* 2002; Purvis *et al* 2005) have been identified as important protective factors for later childhood allergy. Other exposures have also been suggested as potential risk factors, though evidence for them is not clear: these include gender, parental education, birth weight, gestational age, socioeconomic status, maternal body mass index (BMI) at birth, parity, season of birth, cesarean section, and miscarriages (Cook & Strachan 1997; Devereux *et al* 2002; Kuyucu *et al* 2004; Purvis *et al* 2005; Hagendorens *et al* 2005; Piippo-Savolainen 2007a; Dietert & Zelikoff 2008; Metsälä *et al* 2008). There is also the suggestion that these perinatal factors may be differentially related to different allergic phenotypes. For instance while day care attendance in early life was a protective risk factor for atopic sensitization, it was a risk factor for wheezing in children (Hagendorens *et al* 2005).

2.3 Diet in the development of allergies and asthma in childhood

In 1994, Seaton and colleagues proposed the hypothesis that diet may play a role in the rising prevalence of allergies and asthma in industrialized societies (Seaton *et al* 1994). That hypothesis was on the premise that the increase in prevalence of aller-

gies and asthma paralleled changes in dietary intake. Ruling out that the rise may be a consequence of a more toxic or allergenic environment, Seaton and colleagues proposed that the change from a more traditional diet to more processed foods characterized by long storage times and transportation may in part explain the rise. Thus, it was hypothesized that the reduction in consumption of the foods which are important sources of antioxidant nutrients might have played a role in the increasing prevalence of allergies (Seaton *et al* 1994; Devereux 2007). Consequently, the decline in lung antioxidant defenses might have induced an increase in airway damage and inflammations, which are key characteristics of allergic phenotypes and asthma (Seaton *et al* 1994; Fogarty & Britton 2000; Devereux 2005; Devereux 2007; Litonjua 2008).

The proposal by Seaton and colleagues was followed by that of Black and Sharpe in 1997, in which they observed that changes in the intake of dietary fats might also explain the increase in allergies and asthma (Black & Sharpe 1997). Particularly, they highlighted that there has been a decrease in the intake of saturated fats and oily fish, the later being a rich source of long chain n-3 polyunsaturated fatty acids (PUFA), and an increase in the intake of n-6 PUFA. This trend, they observed, coincided with the increase in allergies and asthma. A suggested mechanism was that, as precursors for pro-inflammatory eicosanoids, n-6 PUFA may increase the risk of allergies and asthma, whereas n-3 PUFA, which are anti-inflamatory mediators, may be protective (Black & Sharpe 1997; Devereux 2005; Devereux 2007; Litonjua 2008). Therefore, the imbalance of the n-6:n-3 PUFA ratio may lead to the suppression of the T-helper cell 1 (Th1) differentiation and promotion of the Th2 phenotype, ultimately resulting in allergic disorders and asthma (Black & Sharpe 1997; Devereux 2007).

Since these proposals were made, several observational studies have been investigating the association between, particularly, antioxidant and lipid status in children and the occurrence of allergies and asthma. Considering that allergic disorders may have their origins in early life, even before the child is born, particular attention has focused on examining the relation of prenatal and early life dietary exposures to allergies and asthma in childhood (Devereux 2007; Litonjua 2008; de Vries &

Howie 2009). Establishing the role of early life diet in the development of allergies and asthma in childhood raises the potential for early life dietary intervention to prevent allergic disorders (Devereux 2007).

2.3.1 Assessment of dietary intake in studies investigating the role of diet during pregnancy and early life in the development of allergies and asthma in childhood

In epidemiologic studies on the association between diet and disease, several methods are used to assess dietary intake (Willet 1998). The prominent among these methods include 24-hour recall, food records, food frequency questionnaire (FFQ), and measurement of nutrient status using biomarkers. The 24-hour recall involves an interview conducted by a trained interviewer in which the foods consumed during the past 24 hours are probed. The interview can be face to face or on phone (Willet 1998; Litonjua 2008). In the food records method the subjects prospectively record the foods they consume on one or more days. Sometimes photographs of the food items can be provided for assistance. The quantities of the foods can be measured by weighing, using some common household utensils or by using food portion size booklets. Seasonal variations and long term dietary intake may not be captured by using single 24-hour recall and the food records, and such limitations can be minimized by assessing multiple days (Willet 1998; Litonjua 2008).

The FFQ is the most widely used dietary assessment method in epidemiologic studies among adults. Usually it consists of a list of most frequently used foods representing the study population dietary patterns. The subjects are then asked to report how often they eat the specified foods and food groups, and sometimes includes supplements and the quantities of intake. The FFQ provides information on a large number of foods eaten. Individual nutrient intakes can be calculated from the frequency data (Willet 1998; Litonjua 2008). However, the development of the FFQ is expensive, but usually less expensive than other dietary assessment methods at the overall cost. It also always needs to be validated for each population where it is used.

Nutrient biomarkers are most times used to validate the information from the other dietary assessment methods, but they are also commonly used in epidemiologic research. Although biomarkers represent the only reliable markers for individual nutrient status in some cases, they sometimes fail to validly reflect nutrient intake, mostly as a result of genetic variation, behavioral factors, intake of other nutrients, and some other environmental influences (Willet 1998; Litonjua 2008). The features, advantages and disadvantages of the described food consumption assessment methods are presented in Table 1. Maximal insight into the relation between nutrient intake and disease risk requires examining the long-term intake from both foods and dietary supplements. For many nutrients, obtaining a highly accurate estimate of individual intake by using required amount of repeated measurements is often beyond practical possibilities. Accordingly, a high number of repeated measurements could, in fact, cause more erroneous reporting (underreporting) and increase the number of dropouts due to high respondent burden. Choosing an appropriate dietary assessment method is a complex decision, but it may base primarily on the objective of the data collection, the level of accuracy sought and the amount of available resources.

2.3.2 The role of maternal diet during pregnancy

There is an increasing interest in the effect of prenatal dietary exposures on the occurrence of allergies and asthma in the offspring. This interest has been strengthened on the believe that, if diet has a role to play in the development of allergies and asthma, early dietary intervention can be initiated to prevent future occurrence (Devereux 2007). During pregnancy, maternal exposures can be transferred to the fetus through the placenta, thereby shaping the immunological responses of the fetus to fetal allergen exposures before birth (Warner 2004). Such reactions have been indicated to initiate allergic sensitization (Devereux 2002), which may consequently lead to allergic disorders.

Table 1 Features, advantages and disadvantages of the most common food consumption assessment methods in epidemiologic studies[1]

Method	Features	Advantages	Disadvantages
24-hour recall	Probes about foods consumed during the past 24 hours	▪Reasonably precise due to recent memory. ▪Usually involves low participant burden. ▪High participation rate often achieved. ▪All possible foods and their combinations can be recorded.	▪Needs multiple days to reflect long-term dietary intakes. ▪Require well-trained interviewers.
Food records	Subjects prospectively record the foods they consume on one or more days.	▪Record relatively precise dietary intakes over several days. ▪Minimizes bias and recall error as a consequence of its prospective nature.	▪May entail high subject motivation and an adequate level of education. ▪Unless repeated, unable to capture long-term dietary intakes. ▪Subjects may change diet due to recording burden ▪Recording intake may affect food choices.
Food frequency questionnaires (FFQ)	Subjects are presented with a list of several foods and are asked to report how often they eat the specified foods and food groups, often includes dietary supplements and the quantities of intake.	▪Suitable for large cohort studies and assessment of long-term dietary intakes. ▪Due to ease of administration, may be repeated to capture changes of intakes over time. ▪Provide information on large number of foods and food groups. ▪Reasonably cheap to enter and analyze.	▪May be context-specific. ▪Most times need to be validated to ensure main foods in the population are captured. ▪Involves recall, sometimes over a long period of time. ▪Requires subject motivation as a result of long list of foods. ▪Development of FFQ is expensive. ▪Needs to be validated in each population used.
Biomarkers	Most times used to validate the information from FFQ. May represent the only reliable markers for individual nutrient status.	▪May represent the most/only precise measure of short-term nutrient status. ▪Objective measures are not subject to biases of self reported data.	▪May be invasive. ▪Usually requires collection of biological samples (blood, hair, nail). ▪May be time-consuming; and usually expensive.

[1]Modified from Willet 1998 and Litonjua 2008

A major challenge in assessing the role of maternal diet during pregnancy in the development of allergies and asthma in the offspring is the lack of knowledge about the critical time point during pregnancy at which maternal dietary intake influences the occurrence of allergies in the offspring. In most of the studies reviewed, maternal diet during pregnancy was assessed at different time points, thereby making it difficult to compare results across studies. As well as it would be preferable to assess maternal dietary intake throughout the pregnancy period, knowing the critical window when maternal diet maximizes the risk of allergies and asthma in the offspring will present a more specific target for research and intervention.

So far, there is no agreement across studies on the potentially important confounding variables needed to be adjusted for in studies relating maternal diet during pregnancy to allergies in the offspring, making the adjustment of known covariates in these studies less systematic. In assessing the quality of studies, a recent systematic review and meta-analysis concluded that the potential biases in the studies ranged from moderate to substantial (Nurmatov *et al* 2011). Consequently, researchers need to address the methodological challenges faced in this area of research.

Although the biological premise for the effect of prenatal dietary exposures on the occurrence of allergies in the offspring remains complex, a number of prospective cohort studies have been investigating the relation of maternal diet during pregnancy to the development of allergies and asthma in the offspring. Although the effect of maternal diet during pregnancy on the development of allergies and asthma has been examined using other observational epidemiological study designs, such as case-control, cross-sectional, and ecological studies, only prospective birth cohort studies are reviewed in this study, considering their advantages over other observational studies in establishing potential causal relationships. In addition, the consideration of only prospective birth cohort studies enables better comparison with the present study. In the prospective studies considered, maternal dietary intake during pregnancy has been considered mainly on three levels: as foods, as nutrients, and as overall dietary patterns. The summary of previous prospective birth cohort studies examining the association between maternal diet during pregnancy and the risk of

allergies and asthma in the offspring are presented in Tables 2-6 (studies arranged in descending order of year of publication). Most of these studies assessed maternal diet using FFQ.

Foods

Among foods (Table 2), increasing maternal consumption of fish, vegetables, apples, total fruits, citrus fruit, dairy products, milk, and cheese have been associated with decreased risk of allergies in the offspring. On the other hand, high maternal consumption of meat, nuts, fat spreads, vegetable oils, celery, citrus fruit, deep-frying vegetables, and raw pepper have been positively associated with the risk of allergies in the offspring.

The evidence provided from these studies indicates that the association between maternal consumption of fish and the risk (protective) of allergies and asthma in the offspring remains the most consistent among the foods. This agrees with the hypothesis that fish, particularly oily fish which is a rich source of n-3 PUFAs, may confer a beneficial effect on the risk of allergies. The role of other foods remains weak and inconsistent. However, a recent systematic review and meta- analysis concluded that, among the foods, the evidence for the beneficial effects of vegetables and fruits are the strongest (Nurmatov *et al* 2011).

Nutrients

Generally, the hypothesis linking nutrients and the occurrence of allergies have mainly concerned the antioxidant nutrients, vitamin D, and fatty acids. The evidence for the role of fatty acids intake during pregnancy in the development of allergies and asthma in the offspring has been limited (Table 3). However, a Japanese cohort has reported an inverse association between maternal intakes of α-linolenic and docosahexaenoic acids and the risk of allergies in the offspring but a positive association with n-6 PUFA and linoleic acid. Another study from the DIPP cohort has

also reported that n-6-to-n-3 fatty acids increased the risk, whereas PUFAs and alpha-linolenic fatty acids decreased the risk of allergies (Nwaru *et al* In press).

Table 2 Role of maternal food consumption during pregnancy in the occurrence of allergies and asthma in the offspring: summary of evidence from prospective observational studies

Reference, country	Length of follow-up	Subjects	Dietary assessment method, period	Outcomes and assessment	Main findings
Erkkola *et al* 2011 (submitted), Finland	5 years	2441 mother-child pairs	A validated FFQ, the 8th month of pregnancy	ISAAC-based asthma, wheeze, allergic rhinitis, and atopic eczema	Consumption of leafy vegetables, maleceous fruits, and chocolate inversely associated with wheeze
Saito *et al* 2010, Japan	4 months	771 mother-child pairs	A diet history questionnaire (DHQ), anytime during pregnancy	Asthma, assessed through maternal-completed questionnaire: not clear of any standard assessment method used	Consumption of meat positively associated with eczema
Miyake *et al* 2010a & 2010b, Japan	2 years	763 mother-child pairs	DHQ, anytime during pregnancy	Wheeze and eczema; measured using the ISAAC questionnaire	Consumption of green and yellow vegetables, citrus fruits, dairy products, milk, and cheese inversely associated with eczema
Willers *et al* 2008, The Netherlands	8 years	2832 mother-child pairs	FFQ, month prior to first prenatal care check	ISAAC-based wheeze and asthma	Consumption of nuts positively associated with wheeze and asthma symptoms
Fitzsimon *et al* 2007, Ireland	3 years	631 mother-child pairs	FFQ	Doctor-diagnosed asthma	Consumption of fruits, vegetables, and oily fish inversely associated with while spreadable fat positively associated with risk of asthma

Willers *et al* 2007, UK	5 years	1253 mother-child pairs	FFQ, at 32-week pregnancy	ISAAC-based wheeze, asthma, eczema, and rhinitis	Consumption of apple inversely associated with wheeze and asthma and that of fish inversely associated with eczema
Romieu *et al* 2007, Spain	6.5 years	462 mother-child pairs	A validated FFQ, anytime during pregnancy	Parental-reported doctor-diagnosed eczema and wheeze, skin prick test for measurement of allergic sensitization	Fish consumption inversely associated with eczema, wheeze, and allergic sensitization
Sausenthaler *et al* 2007, Germany	2 years	2641 mother-child pairs	FFQ, 8th week of pregnancy	Parental-reported doctor-diagnosed eczema and specific IgE antibodies in serum.	Consumption of margarine and vegetable oils positively whereas fish inversely associated with risk of eczema. Celery, citrus fruit, deep-frying vegetables, and raw sweet pepper positively associated with risk of allergic sensitization.

So far, evidence for the role of maternal antioxidant intakes in the occurrence of allergies and asthma in the offspring have been inconsistent (Table 4), but the main finding include a protective effect for vitamin E (Martindale *et al* 2005; Litonjua *et al* 2006; Devereux *et al* 2006; Miyake *et al* 2010a), zinc (Litonjua *et al* 2006; Devereux *et al* 2006), vitamin C (Martindale *et al* 2005), vitamin A (Miyake *et al* 2010b), selenium and iron (Shaheen *et al* 2004). Conversely, supplemental folic acid during pregnancy have been positively associated with the risk of allergies and asthma in the offspring (Håberg *et al* 2008; Whitrow *et al* 2009).

For vitamin D (Table 5), there is a more consistent evidence across studies, showing a protective effect on allergies and asthma (Devereux *et al* 2007; Camargo

et al 2007; Erkkola *et al* 2009; Miyake *et al* 2010a) except for one study from the UK (Gale *et al* 2008) which showed a positive association. That study, however, has been blamed for some methodological shortcomings, including substantial loss to follow-up and unable to control for important confounding variables. In the recent systematic review and meta-analysis, the strongest and consistent evidence for the nutrients was found for vitamins A, D, E and zinc (Nurmatov *et al* 2011).

Table 3 Role of maternal intake of fatty acids during pregnancy in the occurrence of allergies and asthma in the offspring: summary of evidence from prospective observational studies

Study, birth cohort, and country	Length of follow-up	Subjects	Dietary assessment method, period	Outcomes and assessment	Main findings
Nwaru *et al* 2011 (*In press*), Finland	5 years	2441 mother-child pairs	A validated FFQ, 8th month of pregnancy	ISAAC-based allergic rhinitis and atopic eczema	Maternal consumption of butter and higher ratio of n-6-to-n-3 fatty acids positively associated with, whereas dietary PUFAs and alpha-linolenic fatty acids negatively associated with the risk, of allergic rhinitis
Miyake *et al* 2009a, Japan	2 years	763 mother-child pairs	DHQ, anytime during pregnancy	Wheeze and eczema; measured using the ISAAC questionnaire	Intake of α-linolenic and docosahexaenoic acids inversely associated with wheeze and that of n-6 PUFA and linoleic acid positively associated with eczema

Table 4 Role of maternal intake of antioxidants and status during pregnancy in the occurrence of allergies and asthma in the offspring: summary of evidence from prospective observational studies

Study, birth cohort, and country	Length of follow-up	Subjects	Dietary assess-ment method, period	Outcomes and assessment	Main findings
Miyake *et al* 2010a & 2010b, Japan	2 years	763 mother-child pairs	DHQ, anytime during pregnancy	Wheeze and eczema; with ISAAC question-naire	Intake of calcium and vitamin E inversely associated with wheeze, while β-carotene in-versely associated with eczema
Whitrow *et al* 2009, US	5.5 years	490 mother-child pairs at 3.5 years and 423 at 5.5 years	FFQ, folate intake at early and late preg-nancy	Asthma; with maternal-completed postal questionnaire: not clear of any stan-dard assessment method used	Supplemental folate positively associated with childhood asthma at 3.5 years and persis-tent asthma at 5.5 years
Håberg *et al* 2008, Norway	1.5 years	32077 mother-child pairs	Dietary records, 0 to 30-week of pregnancy	Wheeze; with maternal-completed ques-tionnaire: not clear of any stan-dard assessment method used	Intake of folic acid supplements during 1st trimester positively associated with slight risk of wheeze at 18 months
Devereux *et al* 2006, UK	5 years	1861 partici-pants	FFQ, during the 32-week of preg-nancy	Specific IgE (skin prick test) asthma, wheeze, and eczema assessed using the ISAAC questionnaire	Intake of vitamin E inversely associated with wheeze and asthma, and zinc in-versely associated with wheeze, asthma, and eczema.
Martindale *et al* 2005, Aberdeen, UK	2 years	1300 mother-child pairs	FFQ, during the 34-week of preg-nancy	Wheeze and eczema; assessed based on the ISAAC question-naire	Intake of vitamin C positively associated with wheeze and ec-zema; vitamin E in-versely associated with wheeze as well as ec-zema in children of atopic mothers.
Shaheen *et al* 2004, UK	3.5 years	2530 mother-child pairs	Umbilical cord concentra-tions of antioxi-dants and minerals	Wheeze; with maternal-completed ques-tionnaire: not clear of any stan-dard assessment method used	Cord blood selenium inversely associated with wheeze and iron inversely associated with wheeze and ec-zema.

Table 5 Role of maternal intake of vitamin D and status during pregnancy in the occurrence of allergies and asthma in the offspring: summary of evidence from prospective observational studies

Study, birth cohort, and country	Length of follow-up	Subjects	Dietary assessment method, period	Outcomes and assessment	Main findings
Miyake *et al* 2010a, Japan	2 years	763 mother-child pairs	DHQ, anytime during pregnancy	Wheeze and eczema; measured using the ISAAC questionnaire	Dietary vitamin D inversely associated with wheeze and eczema
Erkkola *et al* 2009, Finland	5 years	1669 mother-child pairs	FFQ, 8th month of pregnancy	Asthma, allergic rhinitis, and atopic eczema; with the ISAAC questionnaire.	Total and dietary vitamin D inversely associated with asthma and allergic rhinitis in the offspring
Gale *et al* 2008, UK	9 years	440 mother-child pairs at 9 months and 178 at 9 years	Serum 25-OHD concentration during pregnancy	Eczema by the UK Working Party's diagnostic criteria for atopic dermatitis; asthma by maternal report	Higher 25-OHD levels positively associated with eczema at 9 months and asthma at 9 years
Camargo *et al* 2007, US	3 years	1194 mother-child pairs	FFQ, during the third trimester	Wheeze, eczema, and respiratory infections using The Asthma Predictive Index	Dietary and supplemental vitamin D inversely associated with wheeze
Devereux *et al* 2007, UK	5 years	1212 mother-child pairs	FFQ, during the 32-week of pregnancy	Serum specific IgE, asthma and wheeze by the ISAAC questionnaire	Total and dietary vitamin D inversely associated with wheeze

Dietary Patterns

Recently, due to lack of sufficient evidence supporting the role of maternal intake of individual foods and nutrients in the occurrence of allergies and asthma in the offspring, it has been suggested that the overall dietary patterns may be more important in understanding the role of diet in the development of allergic disorders (Lange *et al* 2010; Miyake *et al* 2011). The argument favoring this proposal is on the premise

that foods and nutrients are usually eaten together and there is a complex interaction among individual dietary factors, thus it is difficult to separate and quantify the effect of single foods and nutrients, especially when such effects are trivial (Hu 2002; Newby *et al* 2004). Two main methods have been used to examine dietary patterns in epidemiologic studies: the hypothesis-based dietary scores and the data-driven techniques (Table 6).

The Mediterranean dietary scores (Chatzi *et al* 2007) and the Alternate Healthy Eating Index (Lange *et al* 2010) represent the two hypothesis-based approaches that have been related to the risk of allergies in the offspring. Only the Mediterranean dietary scores was associated with the risk of allergies in the offspring, with a beneficial effect (Chatzi *et al* 2007). Principal component analysis (PCA) has been the main data-driven technique that has been used in previous studies, and studies utilizing it have so far found no association between the PCA-derived patterns during pregnancy and the risk of allergies and asthma in the offspring (Shaheen *et al* 2009; Lange *et al* 2010; Miyake *et al* 2011). Consequently, Lange and colleagues concluded that individual foods and nutrients may be the most important in understanding the role of diet in the occurrence of allergies.

Table 6 Role of maternal dietary patterns during pregnancy in the occurrence of allergies and asthma in the offspring: summary of evidence from prospective observational studies

Study, birth cohort, and country	Length of follow-up	Subjects	Dietary patterns technique	Outcomes and assessment	Main findings
Miyake *et al* 2011, Japan	2 years	763 mother child pairs	Principal component analysis (PCA); identified 3 patterns: "Healthy", "Western", "Japanese"	Wheeze and eczema; with the ISAAC questionnaire	The "Western" dietary pattern inversely associated with risk of wheeze
Lange *et al* 2010, US	3 years	1376 mother-child pairs	The Mediterranean diet score; the Alternate Healthy Eating Index; and PCA ("Western" and "Prudent" patterns)	Parental-reported wheeze, doctor-diagnosed asthma, eczema, and serum IgE for specific allergic sensitization	None of the dietary patterns was associated with any of the outcomes
Shaheen *et al* 2009, UK	7 years	12008 mother-child pairs	PCA; identified "Health conscious", "Traditional", "Processed", "Vegetarian" and "Confectionery" patterns	Parental-reported asthma, eczema, and wheeze; serum specific IgE antibodies; and pulmonary functions	None of the dietary patterns was associated with any of the outcomes
Chatzi *et al* 2007Spain	6.5 years	460 mother-child pairs	The Mediterranean diet score	Parental-reported wheeze and specific allergic sensitization by skin prick test	Maternal Mediterranean diet score was inversely associated with wheeze and allergic sensitization

2.3.3 The role of diet during infancy

Although a number of studies have been investigating the effect of dietary intake or status during early childhood on the subsequent development of allergies and asthma

later in life, the focus of the present study is on the role of duration of breastfeeding and age at introduction of complementary foods to the infant. For the prevention of allergies in children, international health organizations recommend that the child be exclusively breastfed for the first 4-6 months, after which complementary foods can be introduced (Høst *et al* 1999; Prescott *et al* 2005; Agostoni *et al* 2008; Greer *et al* 2008). This recommendation has been on the basis that the gut-mucosa barrier of the infant is immature, thus has limited capacity to mount immunological defenses against direct stimulation from environmental signals (Holt & Jones 2000; Bailey *et al* 2005). As a result, early introduction of complementary foods to the child has been postulated to instigate allergic sensitization (Newburg 2005). Although few earlier studies supported this proposition, albeit with insufficient evidence (Fergusson *et al* 1990; Kajosaari 1991), the mechanisms underlying the infant's gut maturational processes are, however, not clearly elucidated, making it difficult to conclude whether short duration of exclusive breastfeeding and early introduction of complementary foods induces any allergy-associated defects on the gut-mucosa of the infant (Holt & Jones 2000; Bailey *et al* 2005). On the other hand, it has been suggested that an immune modulation achieved through an early incremental exposure to specific allergenic foods may induced some tolerance to IgE-mediated food allergies in children (Lack 2007). However, the controversy in this area has persisted, and a recent systematic review and meta-analysis concluded that oral tolerance induction seems not to perform better than avoidance in food-allergic children, hence a call for further research to clarify this proposition (Fisher *et al* 2011).

Despite the current recommendation of 4-6 months exclusive breastfeeding, the role of duration of breastfeeding as a preventive strategy for the development of allergies and asthma in children has remained unclear. While some studies have reported that in children with family history of allergy, exclusive breastfeeding for at least 4 months may protect against allergies, particularly atopic dermatitis (Gdalevich *et al* 2001; Ludvigsson et al 2005; Silvers *et al* 2009; Kull *et al* 2010), others have shown no association (Snijders *et al* 2008; Miyake *et al* 2009). The evidence for asthma remains highly controversial, particularly in the long-term (Greer *et al* 2008; Gdalevich *et al* 2001). Recent reviews conclude that the evidence for the

recommended 4-6 months exclusive breastfeeding as a preventive strategy for the development of allergies and asthma in childhood is inconclusive (Duncan & Sears 2008; Yang *et al* 2009). However, one study showed a marginal association between total breastfeeding beyond seven months (alongside use of complementary foods) and a reduced risk of wheeze in 2-year-old children but not with exclusive breast-feeding (Snijders et al 2008).

Emerging evidence from recent prospective birth cohort studies indicates that the recommendation of introduction of complementary foods after the first 4-6 months may lack strong scientific backing (Table 7: studies arranged in descending order of year of publication). While some studies found no evidence supporting this recommendation (Zutavern *et al* 2008; Chuang *et al* 2011), others have shown that late introduction of complementary foods (roughly >4 months) may, in fact, increase the risk of allergies later in childhood (Poole *et al* 2006; Zutavern *et al* 2006; Mihrshahi *et al* 2007; Snijders *et al* 2008; Virtanen *et al* 2010; Hesselmar *et al* 2010; Koplin *et al* 2010; Joseph *et al* 2011), although "late introduction" was variously categorized in the studies. An earlier systematic review concluded that available data (up to 2005) are insufficient to conclude on the role of timing of introduction of complementary foods in the development of allergies and asthma; however, it reported an increased risk of eczema with introduction of complementary foods less than 4 months (Tarini et al 2006).

Nevertheless, it has been suggested that the issue of reverse causality might have influenced these findings (Khakoo & Lack 2004), which implies that parents with family history of allergies or suspecting a potential allergic risk in their infants may modify the child's diet during the first few months of life: therefore, it is such modification, rather than the timing of introduction of complementary foods, that may actually be associated with the risk of allergies in the child. Although some studies failed to take the issue of reverse causality into account in their analyses (Zutavern *et al* 2004; Poole *et al* 2006), others considered it by stratifying the analysis by early allergic risk or family history of allergies (Zutavern *et al* 2006; Zutavern *et al* 2008; Virtanen *et al* 2010; Hesselmar *et al* 2010; Koplin *et al* 2010; Joseph *et al* 2011), while others excluded subjects with early allergic symptoms in the analysis

(Mihrshahi *et al* 2007; Snijders *et al* 2008; Chuang *et al* 2011; Joseph *et al* 2011). Three of these studies found evidence for reverse causality (Zutavern *et al* 2006; Zutavern *et al* 2008; Joseph *et al* 2011), while the rest reported no evidence for it.

Table 7 Role of age at introduction of complementary foods in allergic sensitization and the occurrence of allergies and asthma: summary of evidence from prospective observational studies

Reference and country	Length of follow-up	Subjects *n*	Complementary foods and their categorization in the analysis	Outcomes and assessment	Main findings
Joseph *et al* 2011, USA	3 years	594	Eggs, milk, and peanut (<4; ≥ 4 mo)	Serum specific IgE (milk, egg, and peanut allergens)	Introduction of new foods < 4 mo inversely associated with allergic sensitization
Chuang *et al* 2011, Taiwan	1.5 years	18733	Any food (< 4, 4-6, > 6 mo)	Doctor-diagnosed atopic dermatitis (AD)	Age at introduction of new foods was not associated with AD
Hesselmar *et al* 2010, Sweden	1.5 years	184	Cow's milk products, potatoes, root vegetables, vegetables, meat, fish, and egg (median month)	Serum examinations for allergic sensitization and symptoms of eczema and asthma	Introduction of fish < 10 mo inversely associated with eczema
Virtanen *et al* 2010, Finland	5 years	1302	Thirds-categorized age at introduction of fruits and berries; roots; wheat, barley, rye, and oats; other cereals; cabbages; milk products; fish; meat; egg	ISAAC-based asthma, allergic rhinitis, and atopic eczema	Introduction of oats < 5 mo inversely associated with asthma and introduction of fish ≤ 6 mo with decreased risk of allergic rhinitis
Zutavern *et al* 2008, Germany	6 years	2074	Any food (0-4; 4-6; >6 mo) and food diversity at 4 mo (no foods; 1-2; 3-8 groups)	Parental-reported asthma, eczema, and allergic rhinitis, serum specific IgE	Neither age at introduction of foods nor food diversity was associated with the outcomes
Snijders *et al* 2008, The Netherlands	2 years	2558	Cow's milk products (0-3; 4-6; 7-9; >9 mo); introduction of 'other foods' (3; 4-6; >7 mo)	Serum specific allergic sensitization; parental reported eczema, AD, and wheeze	Introduction of cow's milk products > 6 mo positively associated with risk of eczema and late introduction of 'other foods' positively associated with risk of allergic sensitization

Table 7 (*Continued)*

Reference and country	Length of follow-up	Subjects *n*	Complementary foods and definition in the analysis	Outcomes and assessment	Main findings
Filipiak *et al* 2007, Germany	4 years	4753	Vegetables, cereal, fruit, meat, dairy products, egg, fish, other food products (≤ 4 5-6, > 6 mo), and food diversity	Parental report of doctor-diagnosed and symptomatic eczema	Timing of introduction of new foods was not associated with the outcome
Mihrshahi *et al* 2007, Australia	5 years	516	'Yes' or 'no' answer on whether any new food was given by 3 months	Skin prick test for allergic sensitization and parental-reported eczema and asthma	Introduction of new foods by 3 months inversely associated with risk of allergic sensitization
Zutavern *et al* 2006, Germany	2 years	2612	Any food, vegetables, fruit, cereal, meat products, and dairy products (0-4; 5-6; >6 mo); egg, fish, and 'others'(0-6; >6 mo); and food diversity	Serum specific allergic sensitization. Parental-reported AD	Introduction of foods >4 mo was inversely associated with symptomatic AD
Poole *et al* 2006, USA	Mean 4.7 years	1612	Cereal grains (wheat, barley, rye, oats) and rice cereal categorized as 0-6, ≥ 7 mo	Wheat allergy: wheat-specific IgE in plasma.	Introduction of cereal grains > 6 mo positively associated with risk of wheat allergy
Zutavern et al 2004, UK	5.5 years	642	Any foods, rice (≤ 3, > 3 mo); fruit, vegetables, cereal (≤ 4, > 4 mo); meat, fish (≤ 5, > 5 mo); milk (≤ 6, > 6 mo); egg (≤ 8, > 8 mo)	Parental-reported doctor-diagnosed asthma, eczema and wheeze; skin prick test for atopy	Introduction of egg > 8 mo and milk > 6 mo positively associated with eczema.

3 AIMS OF THE STUDY

The main aim of this study was to investigate the role of maternal diet during pregnancy and the child's diet during infancy in the development of allergies and asthma in childhood. The overall aim of the study was divided into four sub-aims:

a. To investigate the association between maternal diet during pregnancy and specific allergic sensitization in the offspring at 5 years of age (Study I).
b. To study the association between the age at the introduction of complementary foods during infancy and specific allergic sensitization in the offspring at the age of 5 years (Study II).
c. To validate the asthma component of the ISAAC questionnaire based on parental reports against medical registry data from the Social Insurance Institution in 5-year-old children (Study III).
d. To assess the association between maternal intake of antioxidant nutrients during pregnancy and the occurrence of allergies and asthma in the offspring by the age of 5 years (Study IV).

4 MATERIALS AND METHODS

4.1 Subjects and study design (Studies I to IV)

The present study was based on the Type 1 Diabetes Prediction and Prevention (DIPP) Nutrition Study, which falls within the framework of the DIPP Study. The DIPP Study was started as a multidisciplinary population-based cohort study aimed at examining means to predict and prevent the onset of type 1 diabetes in Finland. Newborn infants with HLA-conferred susceptibility to type 1 diabetes from Turku, Oulu, and Tampere university hospitals are recruited and are being monitored at 3-12 month intervals for diabetes-associated antibodies, growth, and environmental exposures (Kupila *et al* 2001).

In September 1996 and October 1997, the DIPP Nutrition Study was started within the DIPP Study in Oulu and Tampere, respectively (Virtanen *et al* 2006). The aim is to study the relation of maternal diet during pregnancy and lactation, and the child's diet during infancy and early childhood to the development of type 1 diabetes, allergies and asthma in childhood. The dietary data during pregnancy and lactation was gathered by means of validated FFQ (Erkkola *et al* 2001), and that of child's diet by means of food records and a special form for recording the age at introduction of different complementary foods. For the allergy component of the DIPP Nutrition Study, the families of the children who took part in the dietary follow-up were invited at the age of 5 years to participate by completing a questionnaire on the child's history of allergic symptoms modified from the ISAAC questionnaire (Asher *et al* 1995; Remes *et al* 1998). Blood sample for the measurement of IgE was also taken from the participants. Recruitment for the study ended in 2004. Between September 2, 1996 and September 2, 2004, a total of 4065 consecutively born children were invited to participate in the study and 3737 (92%) of these participated. Figure 1 describes the flow and participation of subjects in the study.

The study was approved by the local ethical committees and informed consent was obtained from the parents.

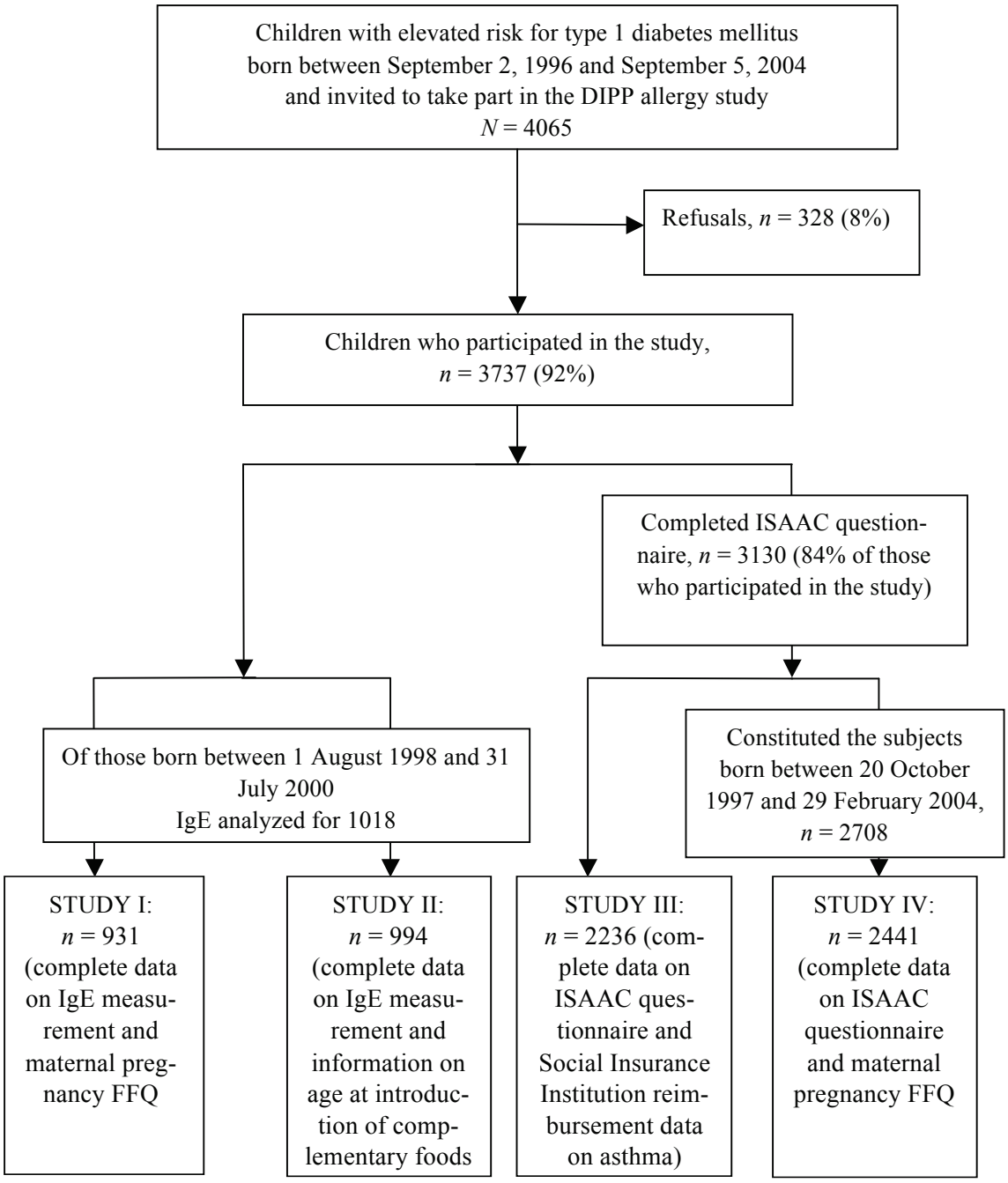


Figure 1. The flow of subjects in Studies I-IV

Subjects in Studies I and II

The subjects in Studies I and II constituted those who participated in the allergy study between August 1, 1998 and July 31, 2000. A total of 1067 children participated, and serum IgE measurements were analyzed for 1018 (87%) of them. For Study I, the final sample size was 931 children, involving those with complete data for IgE measurements and maternal pregnancy FFQ. The final sample for Study II was 994, constituting those with complete data on IgE measurements and information on age at introduction of complementary foods.

Subjects in Study III

The third sub-study validated the asthma component of the ISAAC questionnaire against the Social Insurance Institution reimbursement data on asthma. The validation study was undertaken to assess the validity and utilization of the Finnish ISAAC questionnaire as an assessment tool for asthma in Finnish children for epidemiologic research purposes. Of the 3737 children who participated in the allergy study, 3130 (84%) completed the ISAAC questionnaire on the child's history of allergies and asthma. These children were matched to the Social Insurance Institution reimbursement data using the unique personal identification codes, excluding two children who did not have the identification codes. The Social Insurance Institution data was available up to December 2007. Consequently, only those subjects who completed the ISAAC questionnaire up to the end of June 2007 were included in the validation study. This was done so as to get the Social Insurance Institute reimbursement data 12 months prior to completing the ISAAC questionnaire and 6 months after filling the questionnaire for those who had not started receiving the reimbursement at the time of completing the questionnaire. Altogether 2236 children constituted the sample size for the validation study.

Subjects in Study IV

Of the 3130 subjects who completed the ISAAC questionnaire, maternal FFQ was available for 2441, and these constituted the sample for Study IV.

4.2 Exposure assessment

4.2.1 Maternal diet during pregnancy (Studies I and IV)

Maternal diet during the 8th month of pregnancy was assessed by using a validated 181-item FFQ (Erkkola *et al* 2001). The FFQ was designed specifically for assessing the dietary intakes of pregnant Finnish women. The FFQ was given to mothers after delivery and returned to the study center at the child's 3-month visit. Data was also collected on the type, brand, manufacturer of dietary supplements, including the amount and the weeks of pregnancy during which they were used. The food frequency data was converted to mothers' daily average food and nutrient intakes with software of the National Institute for Health and Welfare, Helsinki, taking into account mothers' choices of types of fats used for food preparations and baking, as well as oils used for salad dressing (Arkkola *et al* 2006). Information on the use of supplements was asked for the whole length of pregnancy. The nutrient contents of the supplements registered as drugs were obtained from the Finnish pharmacopoeia. Information on other supplements was acquired from the National Food Administration, the manufacturer, or both. The research personnel checked the FFQs when returned.

4.2.2 Child's diet during infancy (II)

Assessment of the child's diet was performed by using age-specific (at 3, 6, and 12 months) dietary questionnaire and a follow-up form for recording the age of the

child at the introduction of complementary foods. The age-specific questionnaires assessed information on breastfeeding practice, use of infant formula and cow's milk, use of vitamin preparations, and about the foods the child had consumed up to that age. The questionnaires were returned to the study center after completion at each age while the form for introduction of complementary foods was kept by the parents up to the age of 2 years before being returned to the study center. The questionnaires and the follow-up form were checked by a study nurse at each clinic visit.

4.3 Outcome assessment

4.3.1 IgE measurement (Study I and II)

The IgE concentration at the age of 5 years was analyzed from the blood samples by using ImmunoCAP fluoroenzyme immunoassay (Phadia Diagnostics, Uppsala, Sweden). Measurement was done for the following specific allergens: egg, cow's milk, fish, wheat, house dust mite, cat, timothy grass, and birch. Sensitization to any of the allergens was fixed at ≥0.35 kU/L. In Study I, sensitization to any food and inhalant allergens were used as the endpoints, while sensitization to any food allergen, egg allergen, cow's milk allergen, wheat allergen, and any inhalant allergen constituted the endpoints in Study II.

4.3.2 ISAAC questionnaire (Studies III and IV)

The allergic symptoms (wheeze, allergic rhinitis, and atopic eczema) and asthma assessed in the present study were measured by using a Finnish-modified version of the ISAAC questionnaire. The modification was done by adding other specific questions for each of the disease outcomes in addition to the original ISAAC questions, including, whether the disease has been diagnosed by a doctor; age of the child when the disease was diagnosed; and whether the child has used medication for the

disease during the preceding 12 months. The questionnaire was sent to parents at home when the participating child was 5 years old and returned to the study center after completion and checked by a study nurse. Only the asthma component of the questionnaire was validated in Study III. Asthma, allergic rhinitis, and atopic eczema were the endpoints assessed in Study IV. Asthma was defined as doctor-diagnosed asthma plus either any wheezing symptom during the preceding 12 months or use of asthma medication during the preceding 12 months. Age of the child at asthma diagnosis was determined by the question: 'at what age was asthma diagnosed by a doctor?' Rhinitis was defined as sneezing, nasal congestion, or rhinitis other than with respiratory infections, accompanied by itching of the eye and tearing during the previous 12 months. Age of onset of rhinitis was indicated by the parents at the time of doctor diagnosis. Eczema was defined with a positive answer to the question: 'Has your child ever had atopic eczema?'

4.3.3 Social Insurance Institution database (Study III)

The Finnish Social Insurance Institution houses the anti-asthmatic medication reimbursement database as one of its Special Reimbursement Registers, where prescribed anti-asthmatic medications are recorded (Klaukka 2001). The reimbursement package for anti-asthmatic medications is 75%, and eligibility is that the child provides a doctor's certificate confirming that asthma has been diagnosed and all criteria have been fulfilled (Metsälä *et al* 2008). Usually the reimbursement package is given only 6 months after the child has used the prescribed asthma medications, so as to exclude children having symptoms related to other illnesses apart from asthma. The date of purchase of the medication as well as the personal identification code of each child is recorded in the database.

For the validation of the ISAAC questionnaire, asthma was defined in the Social Insurance Institution database as valid reimbursement (within 12 months before or 6 months after the completion of the ISAAC questionnaire) a purchase of at least one of the following medications: inhaled corticosteroids (R03BA01, R03BA02,

R03BA05); inhaled corticosteroids in fixed combination with long-acting beta agonists (R03AK06, R03AK07); or leukotriene receptor antagonists (R03DC03).

4.4 Assessment and definition of confounding factors

The sociodemographic and perinatal characteristics adjusted as potential confounders in this study were assessed from (1) structured questionnaire completed by the parents after delivery (child's sex, maternal age, maternal and paternal educational level, and the number of siblings); (2) Medical Birth Registries of Oulu and Tampere University Hospitals (hospital of birth, mode of delivery, gestational age, birth weight, birth length, ponderal index, maternal smoking during pregnancy; and (3) from the ISAAC questionnaire (parental asthma, parental allergic rhinitis, pets at home by 1 year of age, atopic eczema before the age of 6 months).

4.5 Statistical analyses

4.5.1 General descriptive analyses (Studies I-IV)

In Studies I, III and IV the Pearson X^2 test was used to analyze the descriptive data: it was used to relate the background characteristics of the participants to the outcomes in Studies I and III, while in Study IV it was used to examine the differences between participants in the allergy study and non-participants with regard to the background characteristics. Means and frequency distributions were in addition used in Studies I and IV. In Study II, descriptive data were analyzed by using Mann-Whitney *U* test and Kruskal-Wallis test, to assess the median duration of exclusive breastfeeding by the background characteristics of participants.

4.5.2 Validation of the ISAAC questionnaire (Study III)

The validation of the ISAAC questionnaire was performed by computing the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The Youden's index (sensitivity + specificity – 1) was used to estimate the overall validity of the questionnaire. The Youden's index facilitates the comparison of validity estimates across populations (Youden 1950). The validity estimates were accompanied with their 95% confidence intervals (CI).

4.5.3 Logistic regression and Cox proportional hazards regression (Studies I, II, IV)

Logistic regression was the main modeling technique used to study the associations between the dietary exposures and allergic sensitization in Studies I and II, and the atopic eczema endpoint in Study IV. Cox proportional hazard regression was used in Study IV for the asthma and allergic rhinitis endpoints.

In Study I, maternal dietary intake was considered both at the food and nutrient levels. The dietary variables were first logarithmically transformed: the nutrients were in addition energy-adjusted using the Willet residual method (Willet 1998) while total energy was included in the models containing foods. In the models, the dietary variables were divided into quarters, the two middle quarters then combined and used as the reference category.

In Study II, age at introduction of complementary foods was divided into thirds. As a first model, each of the complementary foods was examined separately in relation to the endpoints. Those that achieved statistical significance ($P < 0.05$) were included in a backward stepwise logistic regression: the cereals were studied together due to high correlation among them; then the significant ones were studied with other foods. The foods that achieved statistical significance in the stepwise logistic model were adjusted for the potential confounders. The subgroup of children with parental history of allergy (any of the parents with asthma or allergic rhinitis) was studied separately to assess the potential for reverse causality.

In Study IV, total and dietary antioxidants were standardized and their Z-scores were used in their continuous forms in the analysis, adjusting for total energy. Supplemental antioxidants were dichotomized into consumers and non-consumers without adjustment for energy intake.

In Studies I, II, and IV, the following *a priori* defined potential confounders were adjusted in the multiple regression models: sex of child, season of birth (winter, spring, summer, autumn), hospital of delivery (northern or southern Finland), number of siblings (none, one, two or more), maternal age (< 25, 25-29, 30-34, ≥ 35 years), maternal basic education (less than high school, high school graduate), maternal smoking during pregnancy (no, yes, no information), parental asthma (no, yes, no information), parental allergic rhinitis (no, yes, no information). Other confounding factors adjusted for include gestational age at birth (in quartiles) (Studies I and IV); mode of delivery (vaginal, forceps or suction, cesarean section) (Studies II and IV); ponderal index (in quartiles) (Study II); pets at home during the first year of life (no, yes) (Studies II and IV); and atopic eczema by 6 months of age (no, yes) (Study IV). Selection of the confounding variables was based on their relations with the exposure and/or the outcome in the present cohort and on earlier findings.

5 RESULTS

5.1 Basic characteristics of the study population (Studies I to IV)

Of the 2236 children who participated in the validation of the ISAAC questionnaire, 4.5% had valid special reimbursement for anti-asthmatic medication, and 4% had asthma according to the Social Insurance Institution asthma definition. Using the two primary ISAAC combined questions, asthma was found in 6.2% and 6% of the children according to the first and second questions, respectively (Table 8). Higher prevalence of asthma according to both the Social Insurance Institution and ISAAC definitions was seen among boys and children with parental history of asthma and rhinitis (data not shown). Of the participants in Studies I and II (N = 1018), 30% was sensitized to any allergen, 23% to any inhalant allergen, and 17% to any food allergen. Among inhalant allergens, birch (15%) was the most sensitized and cow's milk (12%) among food allergens. In Study IV, of the 2441 children, 6%, 15%, and 38% had asthma, allergic rhinitis, and atopic eczema, respectively (Table 8).

Table 8 Frequency of the outcomes studied

Outcomes	Frequency, *n* (%)
Study I and II (*N* = 1018); IgE positivity for	
Any food or inhalant allergen	305 (30)
Any food allergen	169 (17)
Egg	95 (9.3)
Cow's milk	119 (12)
Fish	11 (1.1)
Wheat	46 (4.5)
Any inhalant allergen	234 (23)
House dust mite	26 (2.6)
Cat	79 (7.8)
Timothy grass	85 (8.3)
Birch	152 (15)
Study III (*N* = 2236)	
Social Insurance Institution asthma definition	90 (4.0)
ISAAC asthma definition 1[a]	138 (6.2)
ISAAC asthma definition 2[b]	135 (6.0)
Study IV (*N* = 2441)	143 (5.9)
Asthma	359 (15)
Allergic rhinitis	926 (38)
Atopic eczema	

[a]Any wheezing symptom or use of asthma medication during the preceding 12 months *plus* asthma ever.
[b]Any wheezing symptom or use of asthma medication during the preceding 12 months *plus* doctor diagnosed asthma

Among the background characteristics studied, children from Southern Finland and those with no siblings were more likely to be sensitized (Table 9); boys were more likely to have allergic rhinitis and asthma, while duration of gestation < 39 weeks was related to eczema and asthma (Table 10). Children of parents with allergic rhinitis and asthma and those with eczema by 6 months were more likely to have any of the allergic outcomes and asthma (Tables 9 & 10). Children from families with pets by the age of 1 year were less likely to have eczema (Table 10).

Table 9 Distribution of allergic sensitization by background characteristics of the subjects

Characteristic		Sensitization to any food allergen		Sensitization to any inhalant allergen	
	N[1]	%	*P*-value[2]	%	%
Sex			0.676		0.162
Boys	551	16		25	
Girls	467	17		21	
Season of birth			0.985		0.905
Spring (Apr – May)	189	17		22	
Summer (Jun – Aug)	227	17		22	
Autumn (Sep – Nov)	258	17		23	
Winter (Dec – Mar)	344	16		24	
Place of birth			0.393		0.026
Tampere (Southern Finland)	566	18		26	
Oulu (Northern Finland)	452	16		20	
No. of siblings at child's birth			0.309		<0.001
None	428	19		30	
One	326	17		20	
Two or more	245	14		16	
Missing information	19	—		—	
Maternal age (years)			0.275		0.967
< 25	166	19		24	
25 – 29.9	343	19		24	
30 – 34.9	312	15		23	
≥ 35	197	14		22	
Maternal basic education			0.803		0.680
Less than high school	374	17		22	
High school graduate	617	17		24	
Missing information	27	—		—	
Maternal smoking in pregnancy			0.840		0.424
No	909	17		23	
Yes	86	16		26	
Missing information	23	—		—	

Table 9 (*Continued*)

Duration of gestation			0.773		0.600
1. quarter: ≤ 38.9 weeks	260	15		22	
2. quarter: 39.0-39.9 weeks	225	16		21	
3. quarter: 40.0-40.7 weeks	275	17		23	
4. quarter: > 40.7 weeks	255	19		26	
Missing information	3	—		—	
Mode of delivery			0.598		0.532
Normal	889	16		23	
Caesarean section	120	18		21	
Missing information	9	—		—	
Parental asthma			0.052		0.252
No	740	15		24	
Yes	126	24		24	
Missing information	152	17		18	
Parental allergic rhinitis			0.010		0.001
No	299	12		18	
Yes	528	20		28	
Missing information	191	15		17	
Pets at home during the first year			0.417		0.730
No	596	18		25	
Yes	273	16		26	
Missing information	149	—		—	
Total length of breastfeeding			0.921		0.758
<6 months	380	17		23	
≥6 months	614	16		23	
Missing information	24	—		—	
Ponderal index			0.869		0.383
First quarter	251	17		27	
Second quarter	253	15		24	
Third quarter	253	18		21	
Fourth quarter	250	16		21	
Missing information	11	—		—	
Total, *N*	1018	169		234	

[1]Subjects in Studies I & II; [2]*P* from Pearson X^2 test.

Table 10 Distribution of atopic eczema, allergic rhinitis, and asthma by background characteristics of the subjects

Characteristic		Atopic eczema		Allergic rhinitis		Asthma	
	N[2]	%	P-value[3]	%	P-value[3]	%	P-value[3]
Sex			0.857		0.004		0.029
Boys	1272	38		17		7	
Girls	1169	39		13		5	
Season of birth			0.546		0.221		0.252
Spring (Apr – May)	637	39		16		6	
Summer (Jun – Aug)	610	40		16		7	
Autumn (Sep – Nov)	978	37		13		5	
Winter (Dec – Mar)	216	36		16		5	
Place of birth			0.145		0.419		0.718
Tampere (Southern Finland)	1586	40		14		6	
Oulu (Northern Finland)	855	37		15		6	
No. of siblings at child's birth			0.427		0.377		0.345
None	1122	38		16		6	
One	750	40		14		7	
Two or more	546	37		15		5	
Missing information	23	—		—		—	
Maternal age (years)			0.090		0.756		0.296
< 25	361	37		16		8	
25 – 29.9	871	41		15		5	
30 – 34.9	751	39		15		6	
≥ 35	458	34		13		6	
Maternal basic education			0.926		0.262		0.272
Less than high school	834	38		16		6	
High school graduate	1558	38		14		5	
Missing information	49	—		—		—	
Maternal smoking in pregnancy			0.445		0.420		0.149
No	2177	39		15		6	
Yes	29	28		21		0	
Missing information	235	40		12		8	
Duration of gestation			0.004		0.562		0.011
1. quarter: ≤ 38.9 weeks	597	40		13		8	
2. quarter: 39.0-39.9 weeks	565	36		15		7	
3. quarter: 40.0-40.7 weeks	614	43		16		4	
4. quarter: > 40.7 weeks	646	34		15		5	
Missing information	19	—		—		—	

Table 10 (*Continued*)

Mode of delivery			0.335		0.076	0.735
Normal	2117	39		14	6	
Caesarean section	309	36		21	6	
Missing information	15	35		15	7	
Parental asthma			<0.001		0.001	<0.001
No	2004	36		13	5	
Yes	381	49		21	13	
Missing information	56	47		15	3	
Parental allergic rhinitis			<0.001		<0.001	0.001
No	797	29		8	3	
Yes	1512	44		19	7	
Missing information	132	40		15	6	
Atopic eczema by 6 months of age			<0.001		<0.001	<0.001
No	2007	26		12	5	
Yes	384	100		31	11	
Missing information	50	—		—	—	
Pets at home during the first year			0.018		0.288	0.459
No	1647	40		15	6	
Yes	783	35		14	5	
Missing information	11	—		—	—	
Total, *N*	2441	926		359	143	

[1]Subjects in Study IV
[2]*P* from Pearson X^2 test.

5.2 Maternal dietary intakes during pregnancy (Studies I and IV)

In Study I, the mean daily intake of total energy during pregnancy was 11639 (SD 3396) KJ, total fruits 207 (161) g, vegetables and roots 359 (160) g, milk products 871 (405) g, fish products 23 (20) g, cereals 192 (68) g, total fat 109 (37) g, iron 16.8 (5.4) mg, and vitamin D 5.2 (2.8) µg. Twenty-eight and 73% of the women took vitamin D and iron supplements during pregnancy, respectively.

The mean total intake of total energy in Study IV was 11386 (3148) KJ, selenium 85 (25) µg, magnesium 474 (127) mg, calcium 1853 (686) mg, manganese 6.7 (2.5) mg, folate 364 (113) µg, riboflavin 2.9 (1.0) mg, and retinol 861 (669) µg. Supplemental intakes of the antioxidants covered only a small proportion of the total intakes, varying from 0.2% for retinol to 23% for riboflavin. The women did not take supplements containing alpha carotene, alpha and gamma tocopherols during pregnancy. Intake of the other major antioxidants (vitamin A, C, E, zinc, and copper) was similar between women in Studies I and IV (data not shown).

5.3 Breastfeeding and introduction of complementary foods during infancy (Study II)

The median duration of exclusive breastfeeding was 1.8 (range: 0-10) months (Study II). Shorter duration of exclusive breastfeeding was related to being born during the autumn, while mothers ≥ 30 years of age, those with high school education, and those with vaginal delivery were more likely to breastfeed for long. Cow's milk (mainly in the form of cow's milk based formula) was introduced at median age of 2 (range: 0-23.5) months. The first complementary food was introduced at median age of 3.5 (range: 0.7-10) months. The first complementary food introduced was potato, followed, in that order, by fruits and berries, carrots, cabbages, cereals, meat, fish, and eggs. The distribution of age at introduction of complementary foods, categorized in thirds, is presented in Table 12.

5.4 Estimates from the validation of the ISAAC questionnaire (Study III)

In the validation study, each of the two primary ISAAC combined questions gave a sensitivity of 0.98 (95% CI 0.92-0.99) and specificity of 0.98 (0.97-0.98). However, while the NPV for both definitions was 1.00 (1.00-1.00), the PPV was just above average, 0.63 (0.55-0.71) and 0.64 (0.57-0.72) for the first and second definitions, respectively. The Youden's index for both asthma definitions was also high (0.96). Furthermore, the validity estimates of the single asthma questions in the questionnaire were comparable to the combined questions, although these were not of particular interest in this study. Only the question on "any wheezing symptoms during the last 12 months" gave lower but reasonable estimates (sensitivity of 0.88 and specificity of 0.75) than other single questions.

5.5 Association between maternal diet during pregnancy and allergies in the offspring (Studies I and IV)

After adjusting for potential confounding variables, maternal consumption of citrus fruit and total fruits were associated with increased risk of sensitization to inhalant allergens in Study I (i.e. a one-gram increase in total fruits and citrus fruit consumption caused a 36% and 14% increase in sensitization to inhalant allergen, respectively), while intake of dietary vitamin D was inversely associated with sensitization to food allergens (i.e. a 44% decreased risk of sensitization was observed with one-microgram increase in vitamin D intake) (Table 11). Also, in Study I there was a potential but unclear borderline association between quarter-categorized intake of polyunsaturated fatty acids (PUFAs) and inhalant allergic sensitization: both low and high intakes of total PUFAs, n-3 and n-6 PUFAs were inversely associated with sensitization to inhalant allergens. A borderline inverse association was also observed between high total vitamin D (intake from foods and supplements combined) and inhalant allergic sensitization (data not shown).

In Study IV, except for dietary magnesium which was inversely associated with the risk of atopic eczema (showing a 22% decreased risk with one-milligram increase in intake of magnesium), none of the other antioxidants was associated with any of the endpoints. However, before confounder adjustment, maternal intake of dietary vitamin C, folate, and riboflavin was negatively associated with eczema. Also, before adjustment, consumers (compared to non-consumers) of supplemental vitamin C, zinc, selenium, magnesium, and manganese had increased risk of eczema in the offspring. Similarly, supplemental intake of vitamin C, copper, and calcium was positively associated with allergic rhinitis before adjustment. None of the total antioxidant nutrients (dietary plus supplemental) was associated with any of the outcomes. Adjustment for vitamin D, stratifying the results by month of birth and parental allergic history, and redefining the asthma outcome (studying only doctor-diagnosed asthma and using wheezing symptoms as outcomes) did not alter any of the results.

5.6 Association between age at introduction of complementary foods and the risk of allergic sensitization in childhood (Study II)

When adjusted for potential confounders, the risk of sensitization to any food allergen increased with late introduction of potatoes (> 4 months), rye (> 7 months), meat (> 5.5 months), fish (> 8.2 months), and eggs (> 10.5 months). Increased risk of sensitization to egg allergen was associated with late introduction of potatoes, carrot (> 4 months), cabbages (> 5.2 months), oats (> 5.5 months), wheat (> 6 months), rye, meat, and eggs. Sensitization to cow's milk allergen was associated with late introduction of meat, fish, and eggs. Sensitization to wheat allergen was associated with late introduction of potatoes, wheat, rye, fish, and eggs. The risk of sensitization to any inhalant allergen increased with late introduction of potatoes, rye, meat, and fish. Finally, exclusive and total breastfeeding and age of introduction of cow's milk were not associated with any of the outcomes (Table 12).

In the stepwise logistic models, only eggs, oats, and wheat were significantly associated with any food and egg allergens. Eggs and wheat were the most important foods associated with sensitization to cow's milk and wheat allergens, respectively. Potatoes and fish were the significant foods related to any inhalant allergen (data not show). There was no evidence of reverse causality in this study as these results were similar among children with parental history of allergies when stratified by that variable. The results did not also change when adjusted for duration of exclusive breastfeeding.

Table 11 Adjusted[1] associations[2] between maternal dietary intake during pregnancy and the risk of allergies and asthma in the offspring

Dietary variables	Specific allergic sensitization Odds ratio (95% Confidence Interval (CI))		Asthma Hazard ration (95% CI)	Allergic rhinitis Hazard ratio (95% CI)	Atopic eczema Odds ratio (95% CI)
	Any food allergen	Any inhalant allergen			
Total fruits	0.97 (0.77-1.23)	1.36 (1.09-1.70)[3]	---[5]	---[5]	---[5]
Citrus fruit	1.00 (0.92-1.09)	1.14 (1.05-1.25)[3]	---[5]	---[5]	---[5]
Vitamin D	0.56 (0.35-0.91)[4]	0.76 (0.50-1.17)	---[5]	---[5]	---[5]
Magnesium	---[5]	---[5]	0.91 (0.59-1.41)	0.95 (0.76-1.19)	0.78 (0.62-0.97)[4]

[1]The estimates of the unadjusted and adjusted models were similar; therefore only results from the adjusted models are presented here.

[2]Only the dietary variables that achieved statistical significance in relation to any of the respective outcomes are presented in the table. Other foods and nutrients studied in Study I include fruits (total fruits, malaceous fruits, citrus fruits, berries, juices), vegetables and roots, potatoes, pulses and nuts, cereals, wheat, dietary fats (butter and butter spreads, margarine and low fat spreads, oils), fish and fish products, milk and milk products (milk fermented milk, cheese), egg, chocolate and sweets, saturated fatty acids, vitamins A (α-carotene, β-carotene), C, D, E (α –tocopherol, γ-tocopherol), zinc, copper, and iron. In Study IV, other antioxidants studied include Vitamins A (alpha and beta carotenes), C, and E (alpha and gamma tocopherols), zinc, copper, selenium, calcium, manganese, folate, riboflavin, and retinol..

[3]$P < 0.01$

[4]$P < 0.05$

[5]Dietary variable not studied for this outcome.

Table 12 Adjusted[1] associations[2] between age at introduction of complementary foods and specific allergic sensitization at the age of 5 years

Age at introduction of complementary foods	Specific allergic sensitization Odds ratio (95% Confidence Interval)				
	Any food allergen	Egg allergen	Cow's milk allergen	Wheat allergen	Any inhalant allergen[3]
Potatoes					
- 1st third: < 3.10	1.00	1.00	1.00	1.00	1.00
- 2nd third: 3.10-4.00	1.50 (0.93-2.42)	1.51 (0.80-2.87)	1.18 (0.69-2.02)	1.71 (0.71-4.11)	1.30 (0.86-1.95)
- 3rd third: >4.00	2.56 (1.49-4.39)[5]	2.88 (1.43-5.81)[5]	1.75 (0.95-3.21)	3.10 (1.19-8.10)[6]	1.93 (1.20-3.11)[5]
p – value[4]	0.003	0.010	0.180	0.064	0.025
Carrot					
- 1st third: < 3.50	1.00	1.00	1.00	1.00	1.00
- 2nd third: 3.50-4.00	1.15 (0.70-1.90)	1.33 (0.67-2.66)	1.20 (0.69-2.12)	1.18 (0.48-2.88)	1.02 (0.66-1.57)
- 3rd third: >4.00	1.66 (0.97-2.83)	2.33 (1.15-4.75)[6]	1.21 (0.65-2.25)	1.75 (0.69-4.44)	1.37 (0.86-2.18)
p – value[4]	0.134	0.040	0.782	0.434	0.282
Cabbages					
- 1st third: < 4.10	1.00	1.00	1.00	1.00	1.00
- 2nd third: 4.10-5.20	1.00 (0.61-1.65)	0.85 (0.42-1.70)	0.81 (0.46-1.43)	0.81 (0.33-1.98)	1.00 (0.66-1.52)
- 3rd third: > 5.20	1.51 (0.94-2.42)	1.96 (1.06-3.60)[6]	1.12 (0.65-1.93)	1.37 (0.63-3.01)	0.86 (0.56-1.30)
p – value[4]	0.141	0.017	0.524	0.449	0.704
Oats					
- 1st third: < 5.00	1.00	1.00	1.00	1.00	1.00
- 2nd third: 5.00-5.50	0.85 (0.48-1.52)	1.02 (0.44-2.34)	1.01 (0.52-1.97)	0.79 (0.28-2.26)	0.71 (0.43-1.15)
- 3rd third: >5.50	1.82 (0.99-3.37)	2.54 (1.08-5.95)[6]	1.48 (0.72-3.03)	2.20 (0.77-6.33)	1.20 (0.71-2.05)
p – value[4]	0.002	0.002	0.298	0.017	0.021
Wheat					
- 1st third: < 5.00	1.00	1.00	1.00	1.00	1.00
- 2nd third: 5.00-6.00	0.97 (0.58-1.61)	0.86 (0.42-1.75)	0.81 (0.45-1.46)	1.29 (0.45-3.70)	0.89 (0.57-1.38)
- 3rd third: > 6.00	1.49 (0.93-2.37)	1.95 (1.06-3.56)[6]	1.21 (0.71-2.05)	3.66 (1.50-8.94)[5]	1.42 (0.94-2.15)
p – value[4]	0.127	0.018	0.391	0.004	0.066

Table 12 (*Continued*)

Rye					
- 1st third: < 5.60	1.00	1.00	1.00	1.00	1.00
- 2nd third: 5.60-7.00	1.23 (0.74-2.03)	1.34 (0.67-2.71)	1.04 (0.59-1.85)	2.21 (0.80-6.09)	1.06 (0.69-1.61)
- 3rd third: > 7.00	2.30 (1.40-3.75)[5]	3.04 (1.57-5.91)[5]	1.66 (0.96-2.90)	4.82 (1.82-12.74)[5]	1.62 (1.06-2.49)[6]
p – value[4]	0.002	0.001	0.121	0.002	0.047
Meat					
- 1st third: < 5.10	1.00	1.00	1.00	1.00	1.00
- 2nd third: 5.10-5.50	0.82 (0.46-1.48)	0.72 (0.32-1.63)	0.81 (0.41-1.61)	0.29 (0.07-1.30)	0.80 (0.48-1.33)
- 3rd third: > 5.50	1.65 (1.07-2.55)[6]	1.84 (1.06-3.20)[6]	1.47 (0.89-2.42)	2.00 (0.99-4.05)	1.64 (1.12-2.41)[6]
p – value[4]	0.027	0.029	0.178	0.017	0.010
Fish					
- 1st third: < 6.10	1.00	1.00	1.00	1.00	1.00
- 2nd third: 6.10-8.20	1.50 (0.89-2.53)	1.79 (0.91-3.50)	1.42 (0.76-2.66)	2.54 (0.92-7.06)	0.81 (0.53-1.26)
- 3rd third: > 8.20	2.42 (1.48-3.95)[5]	2.01 (1.05-3.86)	2.65 (1.50-4.70)[5]	4.20 (1.60-11.01)[5]	1.51 (1.00-2.28)[6]
p – value[4]	0.002	0.094	0.002	0.014	0.015
Egg					
- 1st third: < 8.10	1.00	1.00	1.00	1.00	1.00
- 2nd third: 8.10-10.50	1.04 (0.60-1.80)	1.03 (0.48-2.22)	1.13 (0.59-2.15)	1.65 (0.58-4.71)	1.03 (0.66-1.62)
- 3rd third: > 10.50	2.02 (1.23-3.32)[5]	2.40 (1.22-4.73)[6]	2.04 (1.14-3.64)[6]	3.14 (1.21-8.18)[6]	1.49 (0.98-2.27)
p – value[4]	0.002	0.004	0.017	0.036	0.093

[1]The estimates of the unadjusted and adjusted models were similar; therefore only results from the adjusted models are presented here.
[2]Only the dietary variables that achieved statistical significance in relation to any of the respective outcomes are presented in the table. Other dietary variables studied but not shown include length of exclusive and total breastfeeding, introduction of cow's milk and fruits and berries.
[3]Any inhalant allergic sensitization was defined as sensitization to any of the following inhalants: house dust mite, cat, timothy grass, and birch.
[4]Overall *P* value for the categories.
[5]$P < 0.01$
[6]$P < 0.05$

6 DISCUSSION

6.1 Summary of findings

The evidence from this population-based prospective birth cohort study shows that the Finnish ISAAC questionnaire was highly valid for parental-reported childhood asthma, giving a near perfect sensitivity, specificity, and negative predictive values, but the positive predictive value was only above average. The overall validity of the questionnaire using the Youden's index was also high, indicating minimal misclassification of asthma. Therefore, for epidemiologic research purposes, the Finnish ISAAC questionnaire is a valid instrument for parental assessment of childhood asthma.

The results of the study also indicate that, independent of adjusted potential confounding variables, maternal consumption of citrus and total fruits during pregnancy was associated with increased risk of sensitization to inhalant allergens in the offspring. Conversely, maternal intake of dietary vitamin D and magnesium was inversely associated with sensitization to any food allergen and atopic eczema in the offspring, respectively. The hypothesized roles of PUFAs and other antioxidant nutrients in the occurrence of allergies were not confirmed in this study.

Finally, the study provides evidence for an increased risk of allergic sensitization with late introduction of complementary foods. Specifically, when all foods were studied together, late introduction of oats (>5.5 mo), wheat (>6.0 mo), and eggs (>10.5 mo) increased the risk of sensitization to food allergens, whereas late introduction of potatoes (>4.0 mo) and fish (>8.2 mo) increased the risk of inhalant allergic sensitization. These results were similar among children with parental history of allergies, demonstrating no evidence for reverse causality in this study.

6.2 Strengths and limitations of the study

One of the virtues of the present study is its prospective design and a well-defined study population, entailing an advantage over other observational epidemiologic study designs in investigating causal relationships. In each respective study, dietary exposures were assessed prior to the measurement of the outcomes. Consequently, the subjects were unselected with regard to the outcomes, excluding the potential for outcome-dependent report of the dietary variables. In addition, the subjects participating in the allergic study were generally similar to those who refused participation and this minimized the potential for selection bias.

Although maternal diet during pregnancy was assessed in retrospect (i.e. up to 3 months after birth), the questionnaire has been validated in exactly a similar situation and has been shown suitable for the assessment of diet of pregnant Finnish women (Erkkola *et al* 2001). In the validation study with a 10-day food records as the reference, the FFQ overestimated food consumption. However, the overall quintile notation was acceptable for most of the foods, fatty acids, and the antioxidants (Erkkola *et al* 2001). For dietary assessment, a 3-month period is reasonable to minimize any substantial impact of recall bias. Although nutrient assessments using biomarkers represent a more precise tool, they are limited by being unable to assess the long-term nutrient status. In this case, the FFQ is beneficial, since long-term and habitual intake can be measured.

The asthma component of the ISAAC questionnaire used in this study was validated with a strict definition for the asthma endpoint, giving a high detection rate, and as such excluding asthmatics that may be a result of symptoms related to other childhood infections. Although there is a noted heterogeneity of allergic ailments in childhood, making it difficult for valid identification and classification of allergies, such difficulties are observed to be particular among children less than 3 years. Thus, in addition to studying 5-year-old children, the questions and definitions for the allergic rhinitis and atopic eczema endpoints were done in this study in accordance with recommended diagnosis and best possible definitions in the general population. Allergic sensitization was defined as measured in serum, presenting an

objective approximate proxy indicator for assessment of allergic status of the child as measured by elevated IgE. Since the elevated IgE was independent of any confirmed allergic symptom, the association found may lack generalizability to clinical allergic outcomes. However, allergen-specific IgE remains an important precursor to the development of allergies in children (Wahn *et al* 1997; Kuyucu *et al* 2004), and has been seen to predict wheezing and asthma up to adolescence in the Finnish population (Piippo-Savolainen *et al* 2007b).

The HLA-conferred susceptibility to type 1 diabetes of the subjects may limit the applicability of the findings to the general population. Type 1 diabetes is a Th1-type disease whereas allergies and asthma are related to Th2 cell types. The immune system components and processes related to the production of Th1 and Th2 remain unclear, thus it is uncertain if type 1 diabetes associated antibodies may have any impact on the occurrence of allergies and asthma (Stene & Joner 2004; Dales *et al* 2005). Furthermore, the subjects in the present study constituted 14% of the general population, and the cumulative incidence of allergies in this study parallel that in the general Finnish population (Remes *et al* 1998; Haahtela *et al* 2008).

Due to lack of data, the diet of the child, except for duration of breastfeeding, was not adjusted alongside that of maternal diet during pregnancy. Maternal diet intake may be a surrogate for that of the child; therefore, disentangling the effect of the diet of the child would clarify the independent effect of maternal diet and vice versa (Sausenthaler *et al* 2007). At present, there is generally no clear agreement on the important confounding variables to adjust for when investigating the role of diet during early life and the occurrence of allergies and asthma in childhood. As a result, there is a possibility for under/overadjustment of factors in the present study, although confounder selection was done *a priori* based on the association between the factors and the exposure/outcomes and based on evidence from previous studies. There is an ongoing attempt to assess the potential confounders that need to be taken into account when studying the effect of diet and allergies and asthma in children.

6.3 Comparison of results with previous findings

The observed direct association between maternal consumption of citrus fruit and allergic sensitization is consistent with a previous German study (Sausenthaler *et al* 2007), but contrast Scottish and Japanese findings of no association with both allergic sensitization and asthma and an inverse association with eczema, respectively (Willers *et al* 2007; Miyake *et al* 2010a). Total fruit intake was in addition directly associated with inhalant allergens in the present study. However, when both citrus fruits and total fruits were included in the same model, the observed association with total fruits was lost, leaving citrus fruits only associated with the outcome. This may indicate that citrus fruits may have an independent association with allergic sensitization in the offspring, having been suggested to possess pro-inflammatory allergic properties (Ahrazem *et al* 2005; Crespo *et al* 2006). Apart from the consumption of apples which has been inversely related to asthma and wheeze (Erkkola *et al* 2011; Willers *et al* 2007) but not with eczema and sensitization (Sausenthaler *et al* 2007), other fruits have generally not been shown to predict the risk of allergies and asthma (Willers *et al* 2007; Sausenthaler *et al* 2007).

Maternal consumption of any of the antioxidants investigated in this study during pregnancy was not associated with the outcomes, except for magnesium, which was inversely related to atopic eczema. Antioxidants are suggested to program the child's allergic susceptibility already *in utero* by inhibiting the oxidative damages caused by free radicals (Seaton *et al* 1994; Prescott *et al* 1999; Li-Weber *et al* 2002; Devereux 2007). Consequently, the association between prenatal intake of antioxidants and the occurrence of allergies in the offspring has been examined in several studies, but results are mostly inconsistent. Vitamin E and zinc, in particular, have been inversely associated with asthma (Devereux *et al* 2006), but not with other allergies. Vitamin C (Litonjua *et al* 2006), iron (Shahen *et al* 2004), and calcium (Miyake 2010) have been associated with the risk of eczema. Supplemental folate has also been associated with asthma. Although the present study did not confirm any of these previous findings, a recent meta-analysis concluded that, among the antioxidants, there is strong evidence for protective effect of vitamin A, vitamin E,

and zinc (Nurmatov *et al* 2011). No previous study has shown any association between magnesium and allergic outcomes in children, therefore further studies would be required to justify the present observation.

Vitamin D was inversely associated with allergic sensitization. A previous DIPP study also showed an inverse association between maternal intake of dietary vitamin D and the occurrence of asthma and allergic rhinitis (Erkkola *et al* 2009). These findings are strongly supported by other prospective cohort studies from the US (Camargo *et al* 2007), UK (Devereux *et al* 2007), and Japan (Miyake *et al* 2009), but contrast another UK study with a 9-year follow-up which found an increased risk of eczema and asthma by 9 months and 9 years, respectively, with high maternal concentration of vitamin D (25-OHD) (Gale *et al*., 2008). However, that study has been blamed for some shortcomings, including substantial loss to follow-up and being unable to adjust for potential confounding factors. Overall, among the prenatal dietary exposures related to the occurrence of allergies and asthma in the offspring, the evidence for vitamin D has remained the most consistent. Despite the assessment of vitamin D mainly with FFQ, it is safe to suggest that a clinical trial will be required to elucidate the putative role of maternal vitamin D during pregnancy in the development of allergies and asthma.

Independent of reverse causality and the adjusted confounders, introduction of oats (> 5 months), wheat (> 6 months), eggs (> 10.5 months) was associated with allergic sensitization in the present study. In another study of the DIPP cohort, early introduction of oats was also associated with a decreased risk of asthma while fish was associated with a decreased risk of allergic rhinitis (Virtanen *et al* 2010). While some recent studies have also found no evidence supporting a protective effect of delayed introduction of complementary foods 4-6 months (Zutavern *et al* 2004; Zutavern *et al* 2008; Chuang *et al* 2011), others have mostly reported results concomitant with the observation in the present study, showing that late introduction of complementary foods (roughly > 4 months) may, increase the risk of allergies in childhood (Poole *et al* 2006; Zutavern *et al* 2004; Zutavern *et al* 2006; Mihrshahi *et al* 2007; Snijders *et al* 2008; Hesselmar *et al* 2010; Koplin *et al* 2010; Joseph *et al* 2011). In most cases, the findings from these previous studies were also independent

of reverse causality, so that the suspicion of modification of infant feeding practices among children with maternal or parental allergic history or those with manifestations of atopic diseases early in life did not, in sum, explain these findings.

These observations seem to contrast current recommendations of exclusive breastfeeding for the first 4-6 months for the prevention of allergies in children (Høst *et al* 1999; Greer *et al* 2008), and contrast the hypothesis that the infant's gut-mucosa barrier is immature, thereby may lack the capacity to mount immunological defenses against environmental allergic stimulations (Holt & Jones 2000; Bailey *et al* 2005). Although the maturational processes of the gut are poorly understood, it has been suggested that incremental exposure to specific allergenic foods may induced some tolerance to IgE-mediated food allergies in children (Lack 2007). However, a recent systematic review and meta-analysis suggests that oral tolerance induction seems not to perform better than avoidance in food-allergic children (Fisher *et al* 2011), hence protracting the controversy in this evidence base.

The evidence for the role of duration of breastfeeding has remained more controversial (Duncan & Sears 2008; Yang *et al* 2009). While some evidence suggest that exclusive breastfeeding for the 4 months may prevent the onset of atopic dermatitis (Gdalevich *et al* 2001; Ludvigsson et al 2005; Silvers *et al* 2009; Kull *et al* 2010), its effect on other allergies and asthma is not clear (Greer *et al* 2008; Snijders *et al* 2008; Miyake *et al* 2009), particularly in the long-term (Greer *et al* 2008). The PROBIT trial failed to find any protective effect of either exclusive or total breastfeeding on the risk of asthma and allergies (Kramer *et al* 2007). There is also the suggestion that total duration of breastfeeding (i.e. breastfeeding in addition to introduction of complementary foods) rather than its exclusivity may associated with allergies (Snijders *et al* 2008). However, further studies are needed to confirm these findings.

The current evidence of the effect of the duration of breastfeeding and timing of introduction of complementary foods on the risk of allergies and asthma has only come from observational epidemiologic studies, despite their potential for bias. However, for ethical reasons (i.e. breastfeeding cannot be randomized), carrying out randomized trials to confirm these findings may not be feasible. Therefore, the best

evidence to judge the role of age at introduction of complementary foods in the development of allergies and asthma in childhood remain to come from observational epidemiologic studies. However, the application of these findings in public health practice may remain challenging considering that early introduction of complementary foods has been associated with increased risk of other childhood chronic diseases, particularly type 1 diabetes (Virtanen et al 2011), contrasting current observations regarding allergies and asthma.

6.4 Considerations for interpretation of findings

In interpreting the evidence from the present study, in line with previous findings, several issues entail consideration. First, the mechanism for the effect of diet during early life on the risk of allergies in children is persuasive, because it is well established that the origin of allergies and asthma is already initiated very early in life, possibly *in utero*. Consequently, there is a great potential that maternal diet during pregnancy may influence the probability that the child will develop allergic disorders through the modulation of the fetal airways. Therefore, evidence favoring the role of diet during pregnancy and early life in the development of allergies and asthma later in childhood presents a good opportunity for nutritional interventions as early as possible.

Second, as earlier noted, apart from the evidence for vitamin D, there have been generally inconsistent reports concerning the role of maternal intake of other dietary exposures in the occurrence of allergies and asthma in the offspring. This inconsistency may result from the fact that most of the studies have measured maternal dietary intake at different time points during pregnancy, giving the possibility that each of the studies may be detecting different associations between maternal dietary intake and the development of allergies in the offspring. As has been suggested, there may be a critical time window when the effect of maternal diet may be optimal to predict the occurrence of allergies and asthma in the offspring. Both dietary intake and the occurrence of allergies and asthma may also be influenced by seasonal varia-

tions (Willet 1998; Purvis *et al* 2005). Therefore, the potential for a critical time window could be pursued by measuring maternal dietary intake throughout the pregnancy period, alternatively using maternal nutrient concentrations. So far, most studies have not met this important requirement. Apart from differences in assessment periods, differences in findings among studies may also be explained by differences in the quantity of maternal dietary intake across studies.

Third, considering the number of tests performed in the present study, chance finding may not be totally ruled out as a possible explanation for the observed results. An extensive scrutiny for associations always carries with it the likelihood for chance occurrence of statistically significant results. However, the magnitude of the results, which persisted even after adjustment for potential confounding variables, makes chance unlikely explanation for the results. In any case, reproducibility of the results remains an important option to rule out any chance finding. On the other hand, the inability to take into account the child's dietary intake makes it less probable that the results may be solely attributed to maternal intake alone. The complex relationship between early diet and allergic outcomes in the child implies that maternal diet should be considered in tandem with that of the child, so as to disentangle the independent role of maternal diet on the risk of allergies in the offspring (Sausenthaler *et al* 2007).

Fourth, because of lack of current evidence supporting the cut-off of 4-6 months for the age at introduction of complementary foods for the prevention of allergies and asthma in children, an ad hoc third categorization of the age at introduction of complementary foods was employed. This procedure avoids outcome-dependent choices of categorization. However, the choice of third categorization resulted in a very narrow time difference between, for instance, the first and third categories for some foods. By implication, the narrow differences may cause the analysis to be less sensitive to detect any association between introduction of foods and allergic sensitization. Despite this, and considering that the median length of exclusive breastfeeding in this study was short (1.8 months), the results showed that a simple interval of delay in the age at introduction of complementary foods was positively associated with allergies. The absolute cut-off of 4-6 months for the age at introduction

of complementary foods that has been employed in most previous studies may be deficient. Like in the present study, the introduction of complementary foods is usually done consecutively for different foods, thus usually not given at the same time. This implies that, due to the varied timing of the age at introduction of different foods, the effect on allergic risk for each food may also be specific to the time that food is introduced. Therefore, using the 4-6-month cut-off may overlook the varied timing of introduction of complementary foods, and may probably under/overestimate the association with allergic risk.

Fifth, the independent association between single nutrients and foods and the occurrence of allergies and asthma remains a complex process since dietary factors are not eaten alone, indicating a complex interaction of effects among nutrients and foods. Consequently, it has been suggested that the overall dietary patterns may be a more optimal option in understanding the role of diet during early life and the occurrence of allergies later in childhood (Lange *et al* 2010; Miyake *et al* 2011). However, apart from the Mediterranean dietary patterns (Chatzi *et al* 2008), other data-driven dietary patterns during pregnancy have not been related to the occurrence of allergies in the offspring (Shaheen *et al* 2009; Lange *et al* 2010). The association between maternal dietary patterns during pregnancy and the occurrence of asthma and allergies in the offspring is currently been undertaken in the DIPP study. Nevertheless, beyond the effects of individual foods and nutrients and the overall dietary patterns, the ultimate role of diet on the risk of allergies and asthma may also be more clearly understood by incorporating the larger built environment, that is, taking into account the processes involved from the production, storage, supply, to the consumption of foods. This perspective, which has not been considered in this area of research, is important, considering that the foundational interest in the role of diet in the occurrence of allergies was provoked by the observation that the increasing prevalence of allergies and asthma coincided with a dramatic change in the diet of the western world (Seaton *et al* 1994). Therefore, the advancing knowledge and current interest in the food-environment interactions presents a good opportunity to comprehensively understand the role of prenatal and early diet and the ultimate risk of allergies in childhood.

7 CONCLUSIONS AND FUTURE IMPLICATIONS

This study provides additional evidence that maternal dietary intake during pregnancy and the child's diet during infancy may play a role in the development of allergies and asthma in childhood. Significant from the results is that the risk for allergic sensitization in the offspring may increase with maternal consumption of citrus fruit during pregnancy, independent of known confounders. On the other hand, intake of vitamin D and magnesium may confer a protective effect against allergies. The hypothesized role of other foods and nutrients were not confirmed in this study. Despite a general inconsistency across studies, the observed protective effect of vitamin D has remained the most consistent across studies among the prenatal nutritional dietary factors that have been related to the occurrence of allergies and asthma in childhood. With the current available evidence, a randomized trial may be required to confirm the potential beneficial effect of vitamin D on the risk of allergies. As this is the first study to report an association between maternal prenatal intake of magnesium and the risk of allergy in the offspring, further studies will be required to understand the putative effect of magnesium on the onset of allergies and asthma.

The optimal length of exclusive breastfeeding for the prevention of allergies and asthma in childhood remains to be determined. For the introduction of complementary foods, it could be concluded that they should not be delayed beyond six months. However, the evidence is still accumulating on when before six months would be the optimal time. Although current findings have mainly emanated from observational epidemiological studies, they, in sum, indicate that there may be need for a careful review of current recommendations on infant feeding for the prevention of allergies and asthma. Ethical concerns may inhibit carrying out randomized controlled trial to confirm these findings, since breastfeeding cannot be randomized.

Future studies on the role of prenatal diet and diet during early life in the occurrence of allergies and asthma in children should endeavor to overcome the major methodological challenges in this area of research. Such improvements should include accurately defining and adjusting for all important confounding covariates; improved assessment of dietary intakes and childhood allergic outcomes, possibly using valid markers; and incorporating the potential influence of seasonal variations in both dietary intake and occurrence of the outcomes.

Above and beyond the effects of individual foods, nutrients, and the overall dietary patterns, there is a promising opportunity for incorporating the food-environment interactions in addressing the question of the role of diet in the development of allergies and asthma in children. Observed changes in the diet of the industrialized world, alongside the increasing prevalence of allergies, prompted the suspicion that diet may play a role in the risk of allergies. Therefore, using a sociological perspective, the role of the food-environment interactions will give a more comprehensive understanding of the role of diet in the ultimate risk of allergies and asthma in children. This could focus on how the processes of food production, processing, and delivery impact on the quality of foods and how this is related to the development of allergic disorders and asthma in the population. Adequate understanding of such environmental modifications can then be addressed through appropriate public policies.

8 ACKNOWLEDGEMENTS

This work was conducted at the School of Health Sciences, University of Tampere, and at the Unit of Nutrition, Department of Lifestyle and Participation, National Institute for Health and Welfare. The cordial support from these stations and the provision of facilities to accomplish this research are hereby acknowledged.

I am greatly indebted to my two supervisors, Professors Suvi M. Virtanen and Minna Kaila, for their expertise and guidance through this thesis process. I came into this research through the advice of Suvi, and through these years she has demonstrated an excellent supervision and a versatile knowledge of the fields of nutrition and epidemiology. I am thankful for all her support and tutorship. Despite the complexities of the field of allergy, Minna was always there right from the beginning to offer the needed guidance and support. She demonstrated a keen knowledge of the field and made life a lot easier for. For this I am grateful for her dedication and supervision. I wish to thank Docent Maijaliisa Erkkola (Maikki) and Professor Jaakko Nevalainen for their cooperation and technical support all through my thesis work. I have had an excellent working relationship with Maikki from when I joined the DIPP group, and she was always a call-point for me in emergencies. I am thankful for her guidance. Jaakko was always the statistical consultant who pointed me to the right direction when the goings got tough in terms of the statistical techniques.

I thank the reviewers of my work Docents Petri Kulmala and Kirsi Laitinen for their dedication and meticulous evaluation of my thesis. Their critisms and suggestions were very valuable. I am indebted to Professors Mikael Knip, Jorma Ilonen, Olli Simell, and Riitta Veijola for their excellent cooperation and serving as co-authors in my papers and giving valuable suggestions for the published articles.

I extend my warmest gratitude to all members of the DIPP Nutrition Study, both in Tampere and in Helsinki. I am particularly grateful to Suvi Ahonen and Carina Kronberg-Kippilä who were very pivotal in nurturing me into the DIPP Nutrition Study when I started my work. Suvi has been very helpful in answering all my questions regarding my work and providing me with every detail needed. I thank her for

her patience even when I come asking the same questions over again. I am thankful to Hanna-Mari Takkinen and Heli Tapanainen for their technical supprt in statistical analyses. I thank Liisa Uusitalo, Mirka Lumia, Päivi Luukkainen, Marianne Prasad, Susanna Kautiainen, Minna Pekkala, Johanna Metsälä, Mirva Koreasalo, Markus Mattila, and Mari Åkerlund for all their support.

I am very thankful to Catarina Ståhle-Nieminen for a cheerful and versatile assistance in every practical task related to my studies here at the School of Health Sciences. Catarina knows where to press the button to get things working, and is ready to go extra miles to ensure that the students are in the comfort zone. Without her my studies would not have smoother. I am grateful to my friends and fellow students whose presence, support and encouragement made things a lot easier: Salam El-Amin, Malkiory Matiya, Chrisitan Amirize, Subas Neupane, David Doku, Sylvia Muyingo, and Proscovia Namujju.

I am greatly indebted to my daughters, Chinomnso and Chinazam, who gave tremendous patience to me even when they needed me most. The joys of their presence made the tough times easier, and were a great antidote for all my stress. I am grateful to my parents and siblings for their ceaseless encouragements and prayers all through this work. Words are not enough to describe their worth. Most of all, I am thankful to God Almighty who gave me the strength in this work.

I am thankful to the Doctoral Programs in Public Health, University of Tampere Foundation, Juho Vainio Foundation, and Yrjö Jahnsson Foundation for providing me with the funding to conduct this research.

9 References

Abbas AK, Murphy KM, Sher A. A functional diversity of helper T lymphocytes. *Nature* 1996; 383: 787-793.

Agostoni C, Decsi T, Fewtrell M *et al*. Comple mentary feeding: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2008; 46: 99-110.

Ahrazem O, Ibanez MD, Lopez-Torrejon G, *et al*. Lipid transfer proteins and allergy to oranges. *Int Arch Allergy Immunol* 2005; 137: 201-210.

Aït-Khaled N, Pearce N, Anderson HR, *et al*. Global map of the prevalence of symptoms of rhinoconjunctivitis in children: The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three. *Allergy* 2009; 64: 123-148.

Arkkola T, Uusitalo U, Pietikainen M, *et al*. Dietary intake and use of dietary supplements in relation to demographic variables among pregnant Finnish women. *Br J Nutr* 2006; 96: 913-920.

Asher MI, Keil U, Anderson HR, *et al*. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; 8: 483-491.

Asher MI, Montefort S, Björkstén B, *et al*. Worldwide trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phase One and Three repeat multicountry cross-sectional surveys. *Lancet* 2006; 368:733-743.

Bailey M, Haverson K, Inman C, *et al*. The development of the mucosal immune system pre- and post-weaning: balancing regulatory and effector function. *Proc Nutr Soc* 2005; 64: 451-457.

Barker DJ. *In utero* programming of chronic disease. *Clin Sci (Lond)* 1998; 95: 115-128.

Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol* 2008; 8: 183-192.

Becker AB. Is primary prevention of asthma possible? *Pediatr Pulmonol* 2000; 30: 63-72.

Björkstén B, Clayton T, Ellwood P, *et al*. Worldwide time trends for symptoms of rhinitis and conjunctivitis: Phase III of the International Study of Asthma and Allergies in Childhood. *Pediatr Allergy Immunol* 2008; 19: 110-124.

Black PN, Sharpe S. Dietary fat and asthma: is there a connection? *Eur Respir J* 1997; 10: 6-12.

Burks AW, Palmer KP. Allergies. In *Encyclopedia of Infant and Early Childhood Development*. 2008; 41-51.

Calder PC, Krauss-Etschmann S, de Jong EC, *et al*. Early nutrition and immunity – progress and perspectives. *Br J Nutr* 2006; 96: 774-790.

Calvani M, Alessandri C, Sopo SM, *et al*. Consumption of fish, butter and margarine during pregnancy and development of allergic sensitizations in the offspring: role of maternal atopy. *Pediatr Allergy Immunol* 2006; 17: 94-102.

Camargo CA, Rifas-Shiman SL, Litonjua AA, *et al*. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. *Am J Clin Nutr* 2007; 85: 788-795.

Chatzi L, Torrent M, Romieu I, *et al*. Mediterranean diet in pregnancy is protective for wheeze and atopy in childhood. *Thorax* 2008; 63: 507-513.

Cook DG, Strachan DP. Parental smoking and prevalence of respiratory symptoms and asthma in school age children. *Thorax* 1997: 52: 1081–1094.

Crespo JF, Retzek M, Foetisch K, *et al*. Germin-like protein Cit s 1 and profiling Cit s 2 are major allergens in orange (*Citrus sinensis*) fruits. *Mol Nutr Food Res* 2006; 50: 282-290.

Csonka P, Mertsola J, Kaila M, *et al*. Regional variation in the diagnosis of asthma among preschool-age children. *Pediatr Allergy Immunol* 2000; 11: 189-192.

Chuang C-H, Hsieh W-S, Chen Y-C. *et al*. Infant feeding practices and physician diagnosed atopic dermatitis: a prospective cohort study in Taiwan. *Pediatr Allergy Immunol* 2011; 22: 43-49.

Dales R, Chen Y, Lin M, Karsh J. The association between allergy and diabetes in the Canadian population: Implications for the Th1-Th2 hypothesis. *Eur J Epidemiol* 2005; 20:713-717.

de Vries A, Howie SEM. Diet and asthma – can you change what you or your children are by changing what you eat? *Pharmacol Ther* 2009; 122: 78-82.

Devereux G. The increase in the prevalence of asthma and allergy: food for thought. *Nat Rev Immunol* 2006; 6: 869-874.

Devereux G. Early life events in asthma – diet. *Pediatr Pulmonol* 2007; 42: 663-673.

Devereux G, Barker RN, Seaton A. Antenatal determinants of neonatal immune responses to allergens. *Clin Exp Allergy* 2002; 32: 43-50.

Devereux G, Seaton A. Diet as a risk factor for atopy and asthma. *J Allergy Clin Immunol* 2005; 115: 1109-1117.

Devereux G, Turner SW, Craig LCA, *et al*. Low maternal vitamin E intake during pregnancy is associated with asthma in 5-year-old children. *Am J Respir Crit Care Med* 2006; 174: 499-507.

Devereux G, Litonjua AA, Turner SW, *et al*. Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am J Clin Nutr* 2007; 85: 853-859.

Dietert RR, Zelikoff JT. Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. *Birth Defects Research (Part B)* 2008; 83: 547-560.

Douwes J, Pearce N. Asthma and the westernization 'package'. *Int J Epidemiol* 2002; 31: 1098-1102.

Duncan JM, Sears MR. Breastfeeding and allergies: time for a change in paradigm? *Curr Opin Allergy Clin Immunol*. 2008, 8:398-405.

Eigenmann PA. Diagnosis of allergy sundromes: do symptoms always mean allergy? *Allergy* 2005; 60 (Suppl. 79): 6-9.

Erkkola M, Karppinen M, Javanainen J, *et al*. Validity and reproducibility of a food frequency questionnaire for pregnant Finnish women. *Am J Epidemiol* 2001; 154: 466–476.

Erkkola M, Kaila M, Nwaru BI, *et al*. Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children. *Clin Exp Allergy* 2009; 39: 875-882.

Erkkola M, Nwaru BI, Kaila M, *et al*. Risk of asthma and allergic outcomes in the offspring in relation to maternal food consumption during pregnancy: a Finnish birth cohort study. *Submitted*.

Fergusson DM, Horwood LJ, Shannon FT. Early solid feeding and recurrent childhood eczema: a 10-year longitudinal study. *Pediatrics* 1990; 86: 541-546.

Fisher HR, du Toit G, Lack G. Specific oral tolerance induction in food allergic children: is oral desensitization more effective than allergen avoidance: a meta-analysis of published RCTs. *Arch Dis Child* 2011; 96:259-264.

Fitzsimon N, Fallon U, O'Mahony D, *et al*. Mothers' dietary patterns during pregnancy and risk of asthma symptoms in children at 3 years. *Ir Med J 2007*; 100(suppl): 27S-32S.

Fogarty A, Britton J. The role of diet in the aetiology of asthma. *Clin Exp Allergy* 2000; 30: 615-627.

Gale CR, Robinson SM, Harvey NC, *et al*. Maternal vitamin D status during pregnancy and child outcomes. *Eur J Clin Nutr* 2008; 62: 68-77.

Gdalevich M, Mimouoni D, David M, et al. Breastfeeding and the onset of atopic dermatitis in childhood: a systematic review and meta-analysis of prospective studies. *J Am Acad Dermatol* 2001, 45:520-527.

Gern JE. Barnyard microbes and childhood asthma. *N Engl J Med* 2011 ; 8 : 769-770.

Georas SN, Guo J, De Fanis U, *et al*. T-helper cell type-2 regulation in allergic disease. *EurRespir J* 2005; 26: 1119-1137.

Grant JA, Horner CC. Allergy: overview. In Laurent GJ, Shapiro SD (eds). *Encyclopedia of Respiratory Medicine*. 200665-72.

Greer FR, Sicherer SH, Burks AW, *et al*. Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, and timing of introduction of complementary foods, and hydrolyzed formulas. *Pediatrics* 2008; 121: 183-191.

Haahtela T, Tuomisto LE, Pietinalho A, *et al*. A 10 year asthma programme in Finland: a major change for the better. *Thorax* 2006; 61: 663-670.

Haahtela T, von Hertzen L, Mäkelä M, *et al*. Finnish Allergy Programme 2008-2018 – time to act and change the course. *Allergy* 2008; 63: 634-645.

Hagendorens MM, Bridts CH, Lauwers K, *et al*. Perinatal risk factors for sensitization, atopic dermatitis and wheezing during the first year of life (PIPO study). *Clin Exp Allergy* 2005; 35: 733-740.

Hesselmar B, Saalman R, Rudin A, *et al*. Early fish introduction is associated with less eczema, but not sensitization, in infants. *Acta Paeditr* 2010; 99: 1861-1867.

Holt PG, Jones CA. The development of the immune system during pregnancy and early life. *Allergy* 2000; 55: 688-697.

Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* 2002; 13: 3-9.

Høst A, Koletzko B, Dreborg S, *et al*, Dietary products used in infants for treatment and prevention of dood allergy: Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. *Arch Dis Child* 1999; 81: 80-84.

Håberg SE, London SJ, Stigum H, *et al*. Folic acid supplements in pregnancy and early childhood respiratory health. *Arch Dis Child* 2009; 94: 180-184.

International Study of Asthma and Allergies in Childhood (ISAAC) Manual, 2nd Edition, Auckland, New Zealand/Münster, Federal Republic of Germany, December 1993.

Johansson SGO, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004; 113: 832-836.

Jones AC, Miles EA, Warner JO, *et al*. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. *Pediatr Allergy Immunol* 1996; 7: 109-116.

Joseph CM, Ownby DR, Havstad SL, *et al*. Early complementary feeding and risk of food sensitization in a birth cohort. *J Allergy Clin Immunol* 2011; 127: 1203-1210.

Kaila M, Rautava P, Holmberg-Marttila D, *et al*. Allergy from infancy to adolescence. A population-based 18-year follow-up cohort. *BMC Pediatrics* 2009; 9: 46.

Kajosaari M. Atopy prophylaxis in high-risk infants: prospective 5-year follow-up study of children with six months exclusive breastfeeding and solid food elimination. *Adv Exp Med Biol* 1991; 310: 453-458.

Kay AB. Allergy and allergies: first of two parts. *New England Journal of Medicine* 2001; 344: 30-37.

Khakoo GA, Lack G. Introduction of solids to the infant diet. *Arch Dis Child* 2004; 89: 295.

Kim SY, Yoon SJ, Jo MW, *et al*. Economic burden of allergic rhinitis in Korea. *Am J Rhinol Allergy*. 2010; 24: e110-3.

Klaukka T. The Finnish database on drug utilization. *Norwegian J of Epidemiol*. 2001;11(1):19-22.

Koplin JJ, Osborne NJ, Wake M, *et al*. Can early introduction of egg prevent egg allergy in infants? A population-based study. *J Allergy Clin Immunol* 2010; 126: 807-813.

Kramer MS, Matush L, Vanilovich I, *et al*. Effect of prolonged and exclusive breast feeding on risk of allergy and asthma: cluster randomized trial. *BMJ* 2007; 335:815.

Kulig M *et al.* (1999). Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *J Allergy Clin Immunol* 103:1173-9.

Kull I, Mele E, Alm J, et al. Breast-feeding in relation to asthma, lung function, and sensitization in young schoolchildren. *J Allergy Clin Immunol*. 2010, 125:1013-1019.

Kupila A, Muona P, Simell T, *et al*. Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. *Diabetologia* 2001; 44:290–297.

Kuyucu S, Saraclar Y, Tuncer A, *et al*. Determinants of atopic sensitization in Turkish school children: Effects of pre- and post-natal events and maternal atopy. *Pediatr Allergy Immunol* 2004: 15: 62-71.

Lack G. The concept of oral tolerance induction to foods. *Nestlé Nutr Workshop Ser Pediatr Program*. 2007; 59: 63-72.

Lange NE, Rifas-Shiman SL, Camargo CA, *et al*. Maternal dietary pattern during pregnancy is not associated with recurrent wheeze in children. *J Allergy Clin Immunol* 2010; 126: 250-255.

Lehtonen EP, Holmberg-Marttila D, Kaila M. Cummulative prevalence of atopic eczema and related skin symptoms in well-baby clinic: a retrospective cohort study. *Pediatr Allergy Immunol* 2003; 14: 405-408.

Litonjua AA. Dietary factors and the development of asthma. Immunol Allergy Clin North Am 2008; 28: 603-629.

Litonjua AA, Rifas-Shiman SL, Ly NP, *et al*. Maternal antioxidant intake in pregnancy and wheezing illnesses in children at 2 y of age. *Am J Clin Nutr* 2006; 84: 903-911.

Li-Weber M, Giasisi M, Trieber MK, Krammer PH. Vitamin E inhibits IL-4 gene expression in peripheral blood T-cells. *Eur J Immunol* 2002; 32:2401-2408.

Ludvigsson JF, Mostrom M, Ludvigsson J, et al. Exclusive breastfeeding and risk of atopic dermatitis in some 8300 infants. *Pediatr Allergy Immunol* 2005, 16:201-208.

Ly NP, Rifas-Shiman SL, Litonjua AA, *et al*. Cord blood cytokines and cute lower respiratory illnesses in the first year of life. *Pediatrics* 2007; e171-e178.

Malmström K, Korhonen K, Kaila M, *et al*. Acute childhood asthma in Finland: A retrospective review of hospital admissions from 1976 to 1995. *Pediatr Allergy Immunol* 2000; 11: 236-240.

Maritz GS, Morley CJ, Harding R. Early developmental origins of impaired lung structure and function. *Early Hum Dev* 2005; 81: 763-771.

Marklund B, Tunsater A, Bengtsson C. How often is diagnosis of bronchial asthma correct? *Fam Pract* 1999; 16: 112-116.

Martindale S, Mcneill G, Devereux G, *et al*. Antioxidant intake in pregnancy in relation to wheeze and eczema in the first two years of life. *Am J Respir Crit Care Med* 2005; 171: 121-128.

Matricardi PM, Yazdanbakhsh M. Mycobacteria and atopy, 6 years later: a fascinating, still unfinished, business. *Clin Exp Allergy* 2003; 33: 717-720.

Metsälä J, Kilkkinen A, Kaila M, *et al*. Perinatal factors and the risk of asthma in childhood--a population-based register study in Finland. Am J Epidemiol. 2008; 168: 170-178.

Mihrshahi S, Ampon R, Webb K, *et al*. The association between infant feeding practices and subsequent atopy among children with a family history of asthma. *Clin Exp Allergy* 2007; 37: 671-679.

Miyake Y, Tanaka K, Sasaki S, et al. Breastfeeding and atopic eczema in Japanese infants: The Osaka Maternal and Child Health Study. *Pediatr Allergy Immunol*. 2009, 20:234-241.

Miyake Y, Sasaki S, Tanaka K, Hirota Y. Consumption of vegetables, fruit, and antioxidants during pregnancy and wheeze and eczema in infants. *Allergy* 2010a; 65: 758-765.

Miyake Y, Sasaki S, Tanaka K, Hirota Y. Dairy food, calcium, and vitamin D intake in pregnancy and wheeze and eczema in infants. *Eur Respir J* 2010b; 35: 1228-1234.

Miyake Y, Okubo H, Sasaki S, *et al*. Maternal dietary patterns during pregnancy and risk of wheeze and eczema in Japanese infants aged 16-24 months: The Osaka Maternal and Child Health Study. *Pediatr Allergy Immunol* 2011; DOI:10.1111/j.1399-3038.1022.01176.x.

Moffatt MF, Gut IG, Demenais F, et al. A large-scale, consortium-based genomwide association study of asthma. *N Engl J Med* 2010 ; 363 : 1211-1221.

Morais-Almeida M, Gaspar A, Pires G, *et al*. Risk factors for asthma symptoms at school age: an 8-year prospective study. *Allergy Asthma Proc* 2007; 28: 183-189.

Neffen H, Gonzalez SN, Fritscher CC, *et al*. The burden of unscheduled health care for asthma in

Latin America. *J Investig Allergol Clin Immunol* 2010; 20: 596-601.

Newburg DS, Innate immunity and human milk. *J Nutr* 2005; 135: 1308-1312.

Newby PK, Tucker KL. Empirically derived eating patterns using factor or cluster analysis: a review. *Nutr Rev* 2004; 62: 177-203.

Nurmatov U, Devereux G, Sheikh A. Nutrients and foods for the primary prevention of asthma and allergy: systematic review and meta-analysis. *J Allergy Clin Immunol* 2011; 127: 724-733.e1-30.

Nwaru BI, Erkkola M, Lumia M, *et al*. Maternal intake of fatty acids during pregnancy and allergies in the offspring. *Br J Nutr* 2011. *In press.*

Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002; 288: 963-972.

Patel SP, Järvelin M-R, Little MP. Systematic review of worldwide variations of the prevalence of wheezing symptoms in children. *Environ Health* 2008; 7: 57.

Pearce N, Aït-Khaled N, Beasley R, *et al*. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax* 2007; 62: 758-766.

Pearce N, Douwes J. The global epidemiology of asthma in children. *Int J Tuberc Lung Dis* 2006; 10: 125-132.

Pekkanen J, Pearce N. Defining asthma in epidemiological studies. *Eur Respir J* 1999; 14: 951-957.

Piippo-Savolainen E, Korppi M. The impact of early passive smoking on lung function in adulthood--a postbronchiolitis study. Acta Paediatr. 2007a; 96: 1860-1861.

Piippo-Savolainen E, Remes S, Korppi M. Does early exposure or sensitization to inhalant allergens predict asthma in wheezing infants? A 20-year follow-up. *Allergy Asthma Proc* 2007b: 28: 454-461.

Platts-Mills TAE, Erwin E, Heymann P, *et al*. Is the hygiene hypothesis still a viable explanation for the increased prevlanece of asthma? *Allergy* 2005; 60 (Suppl. 79): 25-31.

Poole JA, Barriga K, Leung DY, *et al*. Timing of initial exposure to cereal grains and the risk of wheat allergy. Pediatrics 2006; 117: 2175-2182.

Prescott SL, Tang MLK, Australian Society of Clinical Immunology and Allergy. The Australian Society of Clinical Immunology and Allergy position statement: summary of allergy prevention in children. *Med J Aust* 2005; 181: 464-467.

Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; 353:196.

Purvis DJ, Thompson JM, Clark PM, *et al*. Risk factors for atopic dermatitis in New Zealand children at 3.5 years of age. *Br J Dermatol* 2005; 152: 742-749.

Remes ST, Korppi M, Kajosaari M, *et al*. Prevalence of allergic rhinitis and atopic dermatitis among children in four regions of Finland. *Allergy* 1998; 53:682-689.

Remes ST, Pekkanen J, Remes K, *et al*. In search of childhood asthma : questionnaire, tests of bronchial hyperresponsiveness, and clinical evaluation. *Thorax* 2002; 57: 120-126.

Romieu I, Torrent M, Garcia-Esteban R, *et al*. Maternal fish intake during pregnancy and atopy and asthma in infancy. *Clin Exp Allergy* 2007; 37: 518-525.

Saito K, Yokoyama T, Miyake Y, *et al*. Maternal meat and fat consumption during pregnancy and suspected atopic eczema in Japanese infants aged 3-4 months: the Osaka Maternal and Child Health Study. Pediatr Allergy Immunol. 2010; 21(1 Pt 1): 38-46.

Sausenthaler S, Koletzko S, Schaaf B, *et al*. Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 y of age. *Am J Clin Nutr* 2007;85:530-537.

Schoenwetter WF, Dupclay L Jr, Appajosyula S. *et al*. Economic impact and quality-of-life burden of allergic rhinitis. *Curr Med Res Opin* 2004; 20: 305-17.

Scirica CV, Gold DR, Ryan L, *et al*. Predictors of cord blood IgE levels in children at risk of asthma and atopy. *J Allergy Clin Immunol* 2007; 119: 81-88.

Seaton A, Godden DJ, Brown K. Increase in asthma: a more toxic environment or a more susceptible population? *Thorax* 1994; 49: 171-174.

Shaheen SO, Newson RB, Henderson AJ, *et al*. Umbilical cord trace elements and minerals and risk of early childhood wheezing and eczema. *Eur Respir J* 2004; 24: 292-297.

Shaheen SO, Northstone K, Newson RB, *et al*. Dietary patterns in pregnancy and respiratory and atopic outcomes in childhood. *Thoraxi* 2009; 64: 411-417.

Silvers KM, Framptom CM, Wickens K, et al. Breastfeeding protects against adverse respiratory outcomes at 15 months of age. *Matern Child Nutr*. 2009, 5:243-50.

Snijders BE, Thijs C, van Ree R, *et al*. Age at first introduction of cow milk products and other food products in relation to infant atopic manifestations in the first 2 years of life: the KOALA birth cohort study. *Pediatrics* 2008; 122: e115-e122.

Spinossi F, Agea E, Russano A, *et al*. CDA+IL13+ T lymphocytes at birth and the development of wheezing and/or asthma during the 1st year of life. *Int Arch Allergy Immunol* 2001; 124: 497-501.

Stene LC, Joner G, Norwegian Childhood Diabetes Study GroupAtopic disorders and risk of childhood-onset type 1 diabetes in individuals. *Clin Exp Allergy* 2004; 34:201–206.

Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; 299:1259-160.

Tarini BA, Carroll AE, Sox CM, et al. Systematic review of the relationship between early introduction of solid foods to infants and the development of allergic disease. *Arch Pediatr Adolesc Med*. 2006; 160: 502-507.

Virtanen SM, Kenward MG, Erkkola M, *et al*. Age at introduction of new foods and advanced beta cell autoimmunity in young children with HLA-conferred susceptibility to type 1 diabetes. *Diabetologia* 2006; 49:1512–1521.

Virtanen SM, Kaila M, Pekkanen J, *et al*. Early introduction of oats associated with decreased risk of persistent asthma and early introduction of fish with decreased risk of allergic rhinitis. *Br J Nutr* 2010; 103: 266-273.

Virtanen SM, Takkinen H-M, Nevalainen J, et al. Early introduction of root vegetables in infancy associated with advanced ß-cell autoimmunity in young children with human leukocyte antigen-conferred susceptibility to Type 1 diabetes. *Diabet. Med* 2011; 28: 965–971.

van Gool CJAW, Thijs C, Dagnelie PC, et al. Determinants of neonatal IgE level: parity, maternal age, birth season and perinatal essential fatty acid status in infants of atopic mothers. *Allergy* 2004: 59: 961–968.

von Hertzen L, Haahtela T. Reversing trends in the prevalence of asthma. *Allergy* 2005; 60: 283-292.

Wahn U, Bergmann R, Kulig M, *et al*. The natural course of sensitization and atopic disease in infancy and childhood. *Pediatr Allergy Immunol* 1997; 8: S16-20.

Ward DG, Halpin DM, Seamark DA. How accurate is diagnosis of asthma in a general practice database? A review of patients' notes and questionnaire-reported symptoms. *Br J Gen Pract* 2004; 54: 753-758.

Whitrow M, Moore VM, Rumbold AR, *et al*. Effect of supplemental folic acid in pregnancy on childhood asthma: a prospective birth cohort study. *Am J Epidemiol* 2009; 170: 1486-1493.

Willers SM, Devereux G, Craig LCA, *et al*. Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms in 5-year-old children. *Thorax* 2007; 62: 773-779.

Willers SM, Wijga AH, Brunekreef B, *et al*. Maternal food consumption during pregnancy and the longitudinal development of childhood asthma. *Am J Respir Crit Care Med* 2008; 178: 124-131.

Willet W. (ed). Nutritional epidemiology. 2nd edition. New York: Oxford University Press, 1998.

Wordemann M, Polaman K, Diaz RJ, *et al*. The challenge of diagnosing atopic diseases: outcomes in Cuban children depend on definition and methodology. *Allergy* 2006; 61: 1125-1131.

Yang YW, Tsai Cl, Lu CY. Exclusive breastfeeding and incident atopic dermatitis in childhood: a systematic review and meta-analysis of prospective cohort studies. *Br J Dermatol*. 2009, 161:373-283.

Youden WJ. Index for rating diagnostic tests. *Cancer* 1950; 3: 32-35.

Zutavern A, Brockow I, Schaaf B, *et al*. Timing of complementary food introduction in relation to atopic dermatitis and atopic sensitization: results from a prospective birth cohort study. *Pediatrics* 2006; 117: 401-411.

Zutavern A, Brockow I, Schaaf B, *et al*. Timing of complementary food introduction in relation to eczema, asthma, allergic rhinitis, and food and inhalant sensitization at the age of 6 years: results from the prospective birth cohort study LISA. *Pediatrics* 2008; 121: e44-e52.

Zutavern, von Mutius E, Harris J, *et al*. The introduction of complementarys in relation to ashtma and eczema. *Arch Dis Child* 2004; 89: 303-308.